PARASITE EFFECTS ON GROWTH

OF THE MUD WHELK,

COMINELLA GLANDIFORMIS (REEVE, 1874).

A thesis submitted in

partial fulfilment

of the requirements for the

degree of

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Some specimens of the mud whelk, Cominella glandiformis, pictured with the principal means of dissection.

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ABSTRACT.

The mud whelk, *Cominella glandiformis*, from the Avon-Heathcote estuary in Christchurch, is infected by three species of larval digenean parasite. The prevalence of infection in *C. glandiformis* was found to increase with snail length for each parasite species. This introduced the possibility that gigantism could be occurring, and investigation of this formed the study basis.
Differences were apparent between sexes of snail, and juvenile and adult individuals, with respect to both infection and position on the shore. For the analyses used, a combination of length and shell weight was found to be the most effective measure of snail size, but wet and dry tissue weights could also be used.

In order to determine whether gigantism was occurring, a laboratory study was undertaken to investigate any growth rate differences between infected and non-infected snails. The results from this, however, were inconclusive.

The question of whether gigantism could occur in *C. glandiformis* was therefore approached by critically evaluating three hypotheses given in explanation for the phenomenon (Minchella et al. 1985). Histological examination of infected snails was used to distinguish between each hypothesis, and this showed that gigantism was more likely to occur in *C. glandiformis* due to parasite adaptations, rather than transient nutritional surpluses or host adaptations to parasitism.

GENERAL INTRODUCTION.

1. The snail, *Cominella glandiformis* (Reeve, 1874).

The mud whelk, *Cominella glandiformis* (Family Buccinidae, Order Neogastropoda), is common at the Avon-Heathcote estuary in Canterbury, New Zealand, where it is typically found on the surface of mud flats around mid- and low-tide levels. A good general account of the species, including distribution throughout New Zealand, is included in Morton and Miller (1968). It is abundant throughout the estuary, maximum densities of 340 whelks per square metre having been recorded (Jones 1983). A general scavenger of bivalves, numerous *C. glandiformis* will converge on dead animal remains from several feet away with up to sixty feeding off one remains (Bimler 1976, Jones 1983). Principal food items are cockles, top shells, and mud flat snails. As with other members of the genus, *C. glandiformis* is dioecious with internal fertilisation. There has been little research on this snail other than an investigation into 'floating' behaviour and optimal density under laboratory conditions (Bimler 1976).

2. The parasites.

Three larval digenetic trematodes parasitise *C. glandiformis* at the estuary. The only one identifiable to species level is an echinostome *Curtuteria australis* Allison, 1979 (Figure I.1). Most of

this fluke's life-cycle has been described (Allison 1979) - metacercarial cysts are found in the cockle *Austrovenus stutchburyi* and adults occur in the hind-gut of the South Island pied oystercatcher *Haematopus ostralegus finschi*. Little is known of the other two parasite species. Two minor studies on them were conducted for Honours projects at the University of Canterbury (Andrews 1966, Ryburn 1972). The parasites are - (a) a tailed monostomous cercaria (possibly Family Microphallidae) (Figure I.2), and (b) a tailless distome cercaria (Figure I.3). For convenience throughout the course of this thesis each parasite will be referred to as Cercaria 1 (*Curtuteria australis*), Cercaria 2 (tailed monostome), and Cercaria 3 (tailless distome).

Each parasite probably follows the general life-cycle of the Digenea (see Erasmus (1972) for general digenean biology). Eggs, laid by the adult trematode within the gut of a vertebrate, pass out with host faeces. Miracidia hatch from the eggs, seek out a suitable snail host (in this case *Cominella glandiformis*) and penetrate. Miracidia contain either a mother sporocyst or redia, which undergoes an asexual multiplication phase, with numerous daughter rediae (Cercaria 1) or sporocysts (Cercariae 2 and 3) arising from germinal masses within each mother. Cercariae are produced in like manner from each daughter.

In most digeneans both snail hepatopancreas and gonad are parasitised. Infected snails are usually castrated - defined as the total or partial reduction of gamete formation (Malek and Cheng 1974, cited Sullivan et al. 1985). Castration is by physical and/or chemical means (Malek and Cheng 1974).

Host tissue damage is also incurred as mature cercariae free

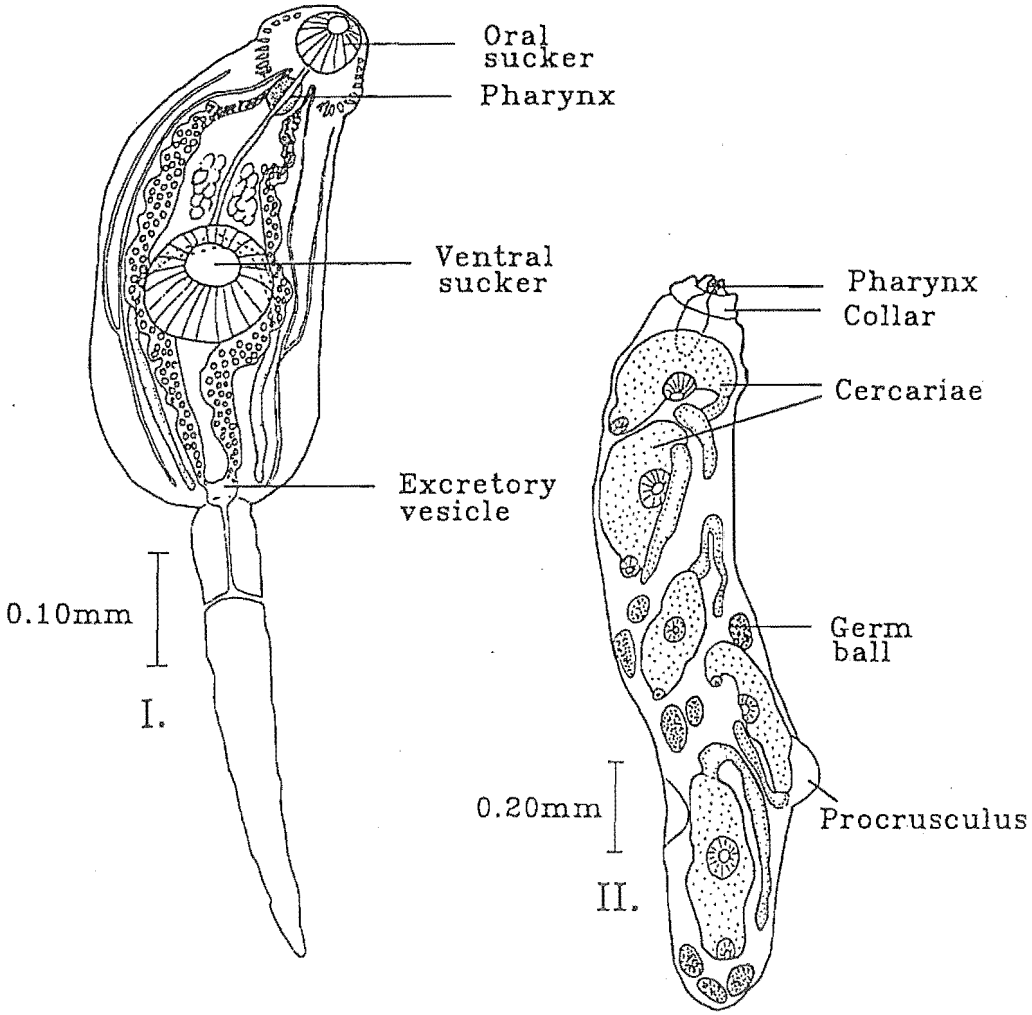


Figure I.1 - Diagrammatical representation of a cercaria and redia of *Curtuteria australis* (Cercaria 1) parasitising *Cominella glandiformis* at the Avon-Heathcote estuary.

I. Cercaria, and II. Redia.

(Both figures from Allison 1979).

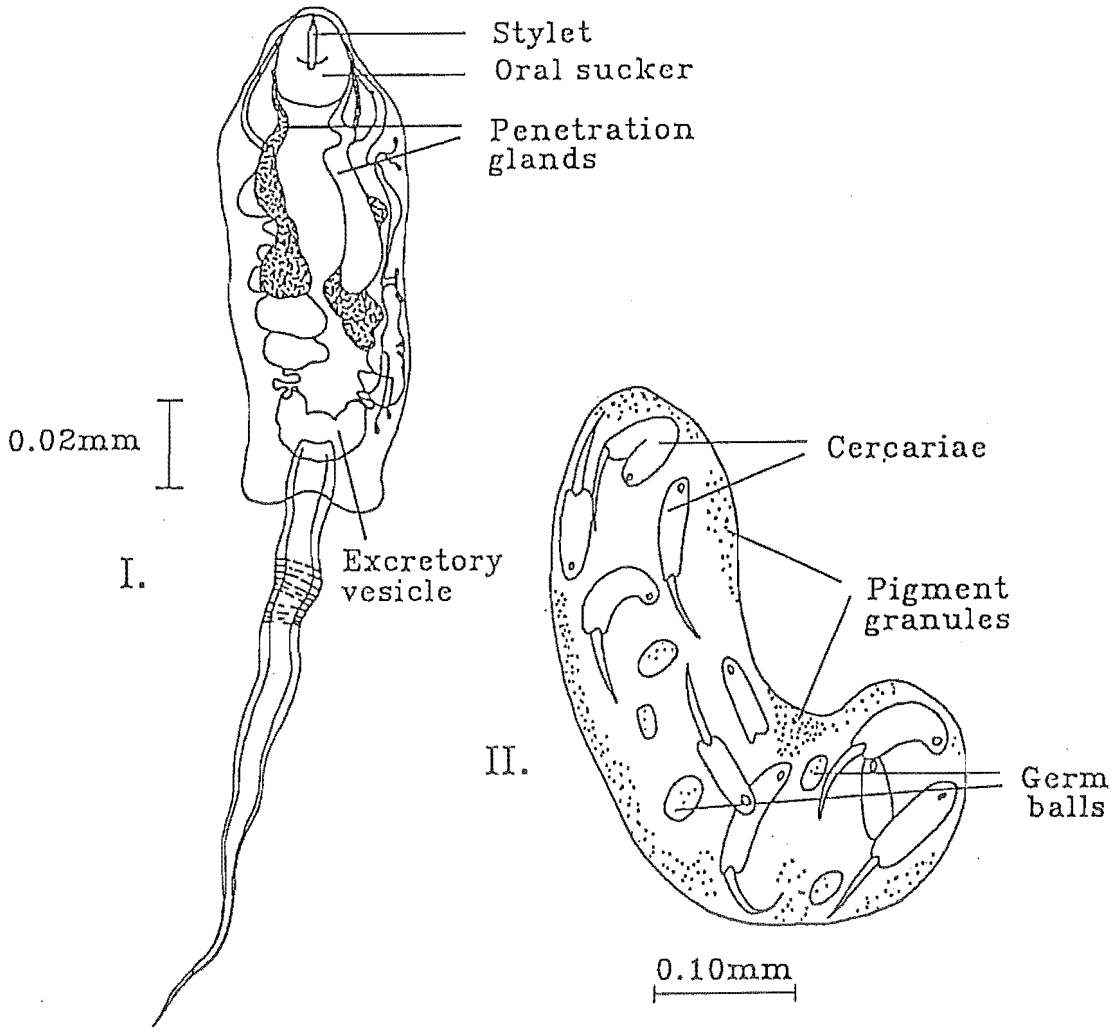


Figure I.2 - Diagrammatical representation of a cercaria and sporocyst from Cercaria 2 infections of *Cominella glandiformis* at the Avon-Heathcote estuary.

I. Cercaria (after Andrews 1966).

II. Redia (after Ryburn 1972).

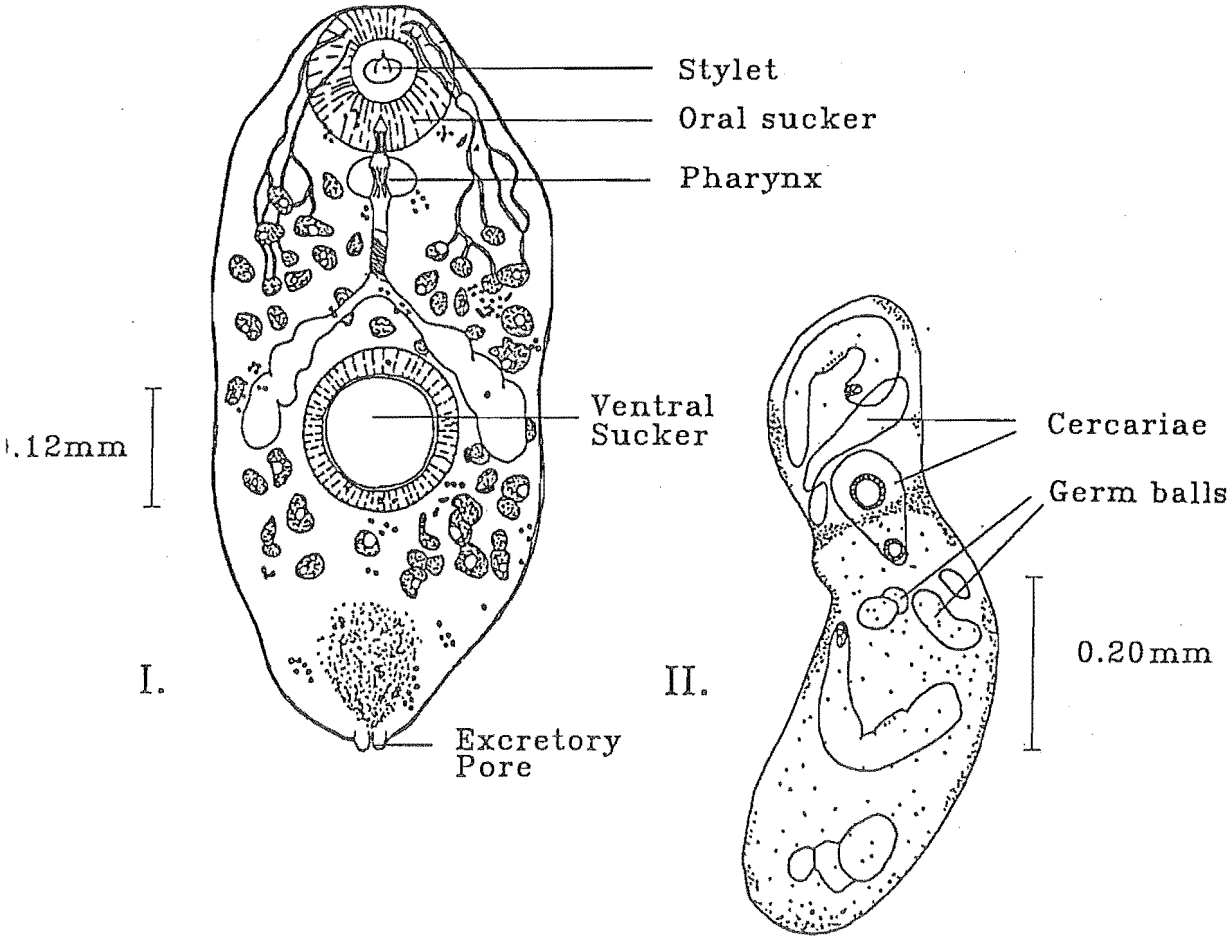


Figure I.3 - Diagrammatical representation of a cercaria and sporocyst from Cercaria 3 infections of *Cominella glandiformis* at the Avon-Heathcote estuary.

I. Cercaria (after Andrews 1966).

II. Sporocyst (after Ryburn 1972).

themselves to leave the snail - the actual means of exit are not known for most flukes but an internal migration route through host tissue and haemolymph is generally assumed (Erasmus 1972). Having left the snail the cercariae then seek a suitable substrate (probably a living organism which acts as a second intermediate host) in which to form metacercariae and await ingestion by the definitive host.

3. The study problem.

A number of phenomena can be associated with parasitic castration. One of these is gigantism - a term applied when it appears that infection is causing a snail to grow larger, perhaps at a faster rate, than normal (Rothschild 1941). "Gigantism" implies a causal relationship between parasite presence and increased rate of host growth but evidence has mostly been indirect, consisting of repeated observations that infection is most prevalent in the largest size-classes of snails (Sousa 1983). There was, before my research began, suggestion of a positive correlation between infection in *Cominella glandiformis* and larger individuals (Ryburn 1972). Gigantism is not, however, the only explanation for correlations such as these. Some alternatives mentioned by Rothschild (1941) include:

(a) Younger (smaller) snails may be unattractive to infective miracidia. Miracidia may use chemical cues for host localisation where such differences exist between adult and juvenile individuals. Older (larger) snails may produce greater quantities of excreta or other attractants increasing the probability of detection and infection by miracidia.

(b) Infection may be lethal to younger snails. Older (larger) snails may be better equipped to buffer the gross effects of parasitism.

(c) Larger (older) snails have had greater time to pick up infection. The probability of infection increases the longer an individual is present in an infective area. Rate of growth may decrease after an individual has attained a certain size so the time period alone could account for heavier infection in larger size classes.

Lysaght (1941) pointed out that no real conclusions can be based on length-size correlations alone, as information on growth rates of uninfected and infected snails of the same initial size is also required.

My study problem was to establish if size/infection correlations existed for *C. glandiformis* and to explore the likelihood of gigantism as an explanation for correlations such as these. Measurements of infection in the field, qualitative assessment of the parasite fauna, and laboratory investigation into the effects on growth of the host are all required for such study.

4. Study Outline.

A combination of four major factors affects the expression of parasite/snail interactions:

i. *Life expectancy and reproductive strategy of the host.* Differing reproductive strategies of snails (perennial or annual) equate with

differences in energy expended on gametogenesis (Minchella 1985). Annual species expend little energy on repair mechanisms but much on reproduction. Perennial species are the reverse, spreading reproduction over a number of years with high levels of maintenance. Parasitic castration releases energy which if not directed towards reproductive effort is available for use elsewhere (e.g. gigantism). Castration of long-lived snails (such as *C. glandiformis*) should result in the release of only small quantities of reproductive energy which could not alone lead to gigantism.

ii. *Host maturity at first infection.* The maturational status (adult or juvenile) of an animal at first infection should determine the amount of energy available from castration, also depending on (i) above. Gonad is not apparent in juvenile snails, so parasitism acts merely to prevent its formation. The lack of reproduction in juvenile snails means that no surplus energy is released due to castration.

iii. *Sex of the host* Calorific differences in expenditure on gametogenesis between the sexes (Baudoin 1975) suggest different amounts of energy are available due to castration.

iv. *Activity level of infection.* Differences may exist between redial and sporocyst infections. Rediae posess a pharynx and actively ingest (physically destroying) host tissue. Sporocysts are apharyngeate (they absorb nutrients across the body wall) and are presumed less capable of physical destruction (Sousa 1983). Castration can be the consequence of either physical or chemical degradation, and may be partial or total. The means and extent of castration can directly affect the quantity of reproductive effort released for use elsewhere.

The extent to which a parasite can nutritionally drain a host is

dependent on factors (i), (ii), (iii) and (iv).

In order to investigate the effects of parasitism on the growth of *C. glandiformis* these factors, along with size/infection correlations and growth rates, also require evaluation. To these ends the work was divided into three sections:

1. **A field study.** Samples were collected from most size-classes of snail for the period of one year, some samples being repeated for additional information for three months of the following year. Data were recorded for all snails as to length, wet and dry tissue weights, shell weight, sex, maturity, and type of infection (if present). This was then analysed for differences between these variables for uninfected snails and those carrying the three types of infection, and for significant correlations between infection and snail size,

2. **A laboratory growth study.** Growth of infected and uninfected individuals was followed for a set period within the laboratory. Snail size (length, weight) was recorded at intervals from the beginning of the study so as to measure growth over time. The data were then analysed for growth rate differences between the experimental groups.

3. **A histological study.** The means and extent of castration by each parasite will affect the degree to which reproductive energy is available for increased growth (Sousa 1982; Minchella 1985). Histological sections of parasitised snails were therefore examined for evidence of reproduction concurrent with infection, and to determine the physical effects of parasitism on host tissue. Experimental anthelmintic trials were undertaken to study the likelihood of host individuals resuming reproduction (via gonadal

regeneration) once infection was eliminated. Data as to this are required in order to interpret some of the general hypotheses, which will be discussed later in the thesis, given in explanation for the phenomenon of gigantism.

Methods, results, and individual discussion for each of these studies are given in three separate sections followed by a general concluding discussion.

CHAPTER 1.

A FIELD STUDY OF *COMINELLA GLANDIFORMIS* AND ITS PARASITE FAUNA FROM THE AVON-HEATHCOTE ESTUARY, WITH EMPHASIS ON THE RELATIONSHIP BETWEEN HOST SIZE AND INFECTION PREVALENCE.

Introduction.

Relatively little is known of *Cominella glandiformis* at the Avon-Heathcote estuary other than brief notes on abundance and distribution (Jones 1983). Also available are two honours projects (Andrews 1966; Ryburn 1972) and one paper (Allison 1979) mainly concerned with identification of the larval parasite fauna to which *C. glandiformis* plays host. As mentioned in the general introduction, the parasites are (a) an echinostome *Curtuteria australis* (Cercaria 1), (b) a tailed monostomous cercaria (possibly Family Microphallidae) (Cercaria 2), and (c) a tailless distome cercaria (Cercaria 3). Ryburn (1972) undertook some experiments attempting to relate parasite prevalence to clumping behaviour of *C. glandiformis* during feeding. No significant differences were found, but suggestion was made of positive correlation between host snail sizes and prevalence of Cercaria 2. Negative correlations were reported with respect to prevalence of Cercaria 3.

For my study purposes a field investigation was initiated to establish what relationships, if any, exist between prevalence of each of the three parasite forms and sizes of their hosts. Previous work on gastropod growth phenomena associated with parasitic castration has

shown that sex (male/female) and maturity (adult/juvenile) also influence rate of growth (ultimately affecting size) (Moose 1963; Zischke and Zischke 1965). In a field study similar to my own, Cannon (1979) attributed positive correlations between infection and host snail size to accumulation of parasites over time, the largest snails also being the oldest. Sousa (1983) also ascribed this explanation to his field results, and in addition found growth rate differences between the sexes.

Methods.

Samples of approximately 200 snails were hand collected from a stretch of the Avon-Heathcote estuary running parallel to Rockinghorse Road on the South New Brigthon Spit (Figure 1.1). Ten samples were taken in all, eight of these during the 1986/87 season (May, July, August, September, November, and December 1986, and January and March 1987), and two the following summer (December 1987, and February 1988). Most of the samples were taken at random from between the high and low water marks, however those for January 1987, December 1987, and February 1988, were collected on a transect line from four different areas, sites 1 through 4 (0-20m, 20-40m, 40-60m, and 60-80m respectively from MHWS), on the shore. This change in procedure resulted from inspection of previous data which suggested size differences between individuals from each shore level. After collection all snails were placed into holding aquaria and processed within one week. The September 1986 sample was unfortunately held

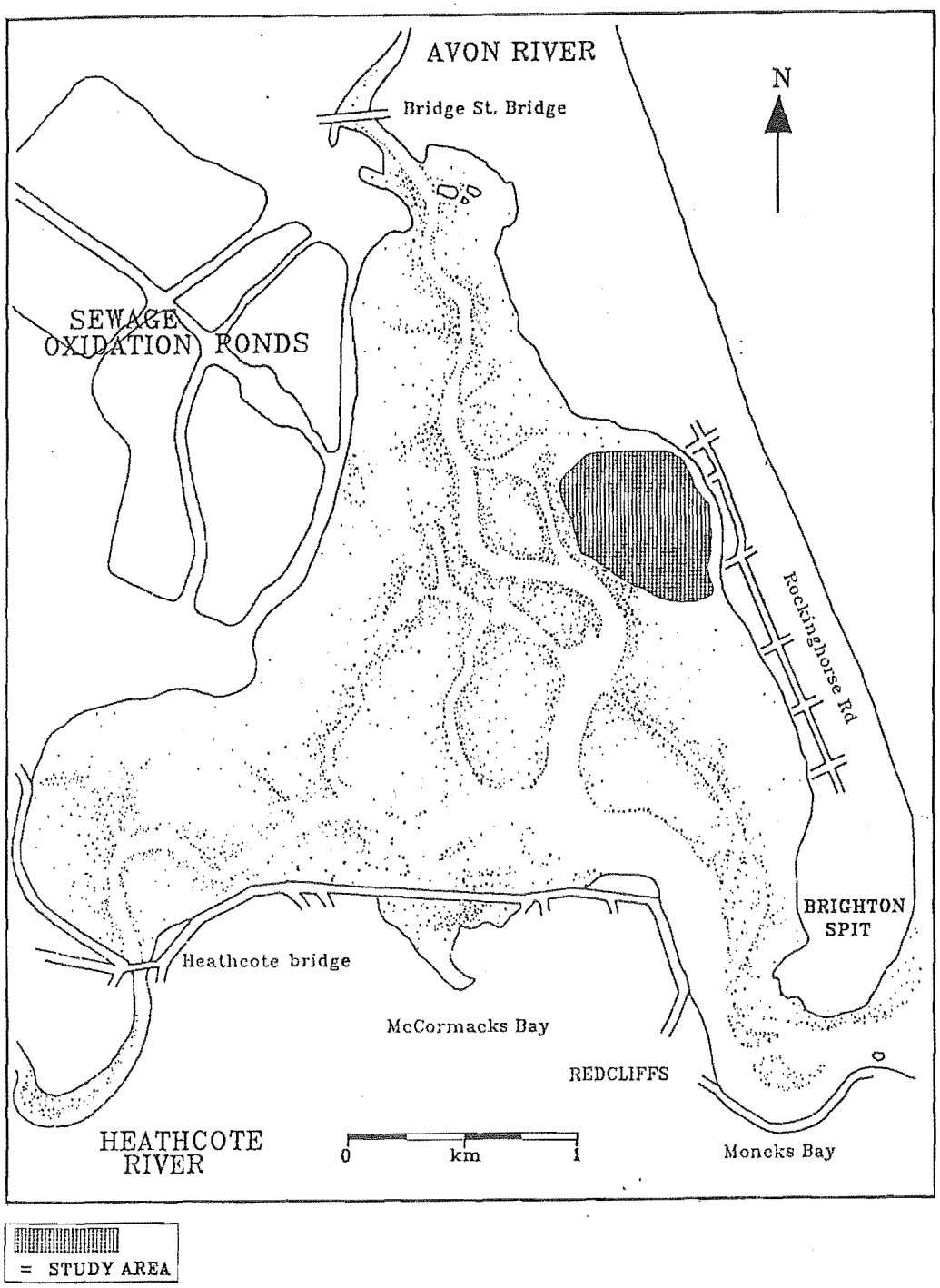


Figure 1.1 - A map of the Avon-Heathcote estuary, Christchurch, New Zealand, showing the area (hatched) used for field sampling of *Cominella glandiformis*. (Figure modified from Jones 1983)

longer than this which resulted in significant weight loss of individuals making data unsuitable for inclusion in the overall analysis.

Length (measured from the top of the spire to the tip of the siphonal canal - see Figure 1.2) was recorded for each snail to the nearest 0.02mm using vernier calipers. Shells were then gently cracked by hammer so as to enable intact removal of the soft body.

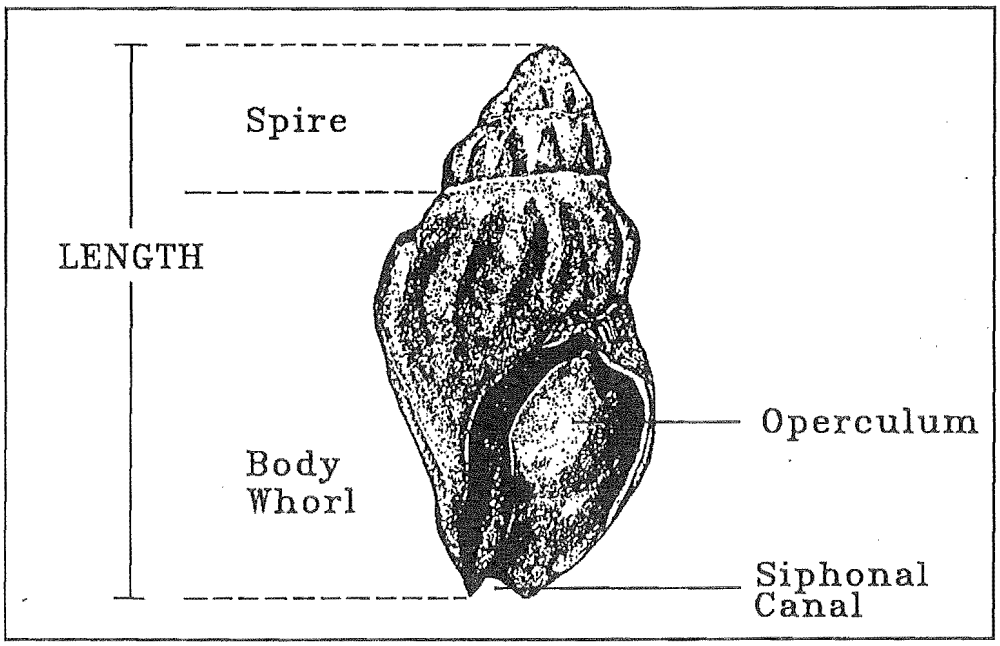


Figure 1.2 - Diagrammatical representation of the shell of *Cominella glandiformis* showing the way in which LENGTH was measured for each animal.

Hepatopancreas and gonad were examined for parasites under the dissecting microscope with the type of parasite, if any, being noted. Sex was established by the presence or absence of a penis to the right

of the head, and maturity (adult/juvenile) determined from the development and condition of ovary (granular and bright orange, covering approximately one third of the dorsal surface of the digestive gland of mature females) or testis (smooth and transluscent white to yellow, almost completely covering the dorsal digestive gland surface of mature males).

Shell fragments and soft body parts of each individual were placed separately into pre-weighed aluminium containers, and wet body tissue weighed. Both shell and tissue were then dried at 60 °C for two days, and re-weighed. All weights were obtained using a Mettler H32 balance, measuring with accuracy to ±0.0003 grams. The aluminium caps were of a uniform size throughout, but during drying lost a constant 0.001g which was corrected for.

Statistical Analysis -

Data were principally analysed using stepwise discriminant analysis (computer package BMDP7M, version 1987) to determine which (if any) of the variables measured could be used as the principle discriminator between groups (i.e. infected and non-infected). This analysis gives a canonical variable - a discriminant function that is a linear combination of one or more of the original variables chosen in such a way as to maximise separation between the groups while minimising the variance within each group (Legendre and Legendre 1983). The original variables are added to the discriminant functions one by one (based on the highest F-to-enter - equivalent to F from a one-way ANOVA) at each step until it is found that adding extra variables does not give significantly better discrimination (Manly 1986). Only those variables with significant F values ($p < 0.01$) are

entered. A U-statistic (Wilks' lambda) is also calculated and can be used as a rough approximation of the proportion of remaining variance after each variable is entered into the discriminant function. Some idea of the power of a single variable as discriminator is gained from its percentage contribution to the total canonical variable (calculated from standardised (by pooled within variances) coefficients for the canonical variable, % SCV hereafter). BMDP also calculates a jack-knifed classification stating the percentage of cases correctly assigned, based on the canonical variable, to their original groups. "Jack-knifing" has the effect of removing bias from the classification by allocating each case to its closest group without using that individual case to help determine the group centre (Manly 1986).

As rate of growth may be affected by sex (male/female) and maturity (adult/juvenile), a multiple analysis of variance (MANOVA) (also on BMDP) was used to gain some measure of how the data varies in relation to these factors. Unlike stepwise discriminant analysis, MANOVA attributes sources of variation within the dependent size (length, tissue wet weight, tissue dry weight, shell weight) variables to each of the independent variables (sex, maturity, and infection). In a three-way factorial analysis such as this the source of variation is sub-divided into three main effects (A, B, C), three first-order interactions (A x B, A x C, and B x C), and one second-order interaction (A x B x C) (Sokal and Rohlf 1981).

Results.

The mean infection prevalences for the total pooled data (N = 1959) (Table 1.1) were 5.77% for Cercaria 1, 12.86% for Cercaria 2, and 9.04% for Cercaria 3. When data were collected along a transect (N = 584), from four sites representing equal sub-divisions to 80m from MHWS, an apparent bias in earlier samples (arising from unequal representation of each site) was shown. Both Cercariae 2 and 3 were under-represented in earlier samples and, as Table 1.2 shows, occurred in 18.93% and 34.32% respectively of snails from site 1 (0-20m from MHWS - i.e. high tide area). Using length as a measure of size, chi-squares on each type of infection versus non-infection between sites were most significant for Cercaria 3 ($X^2 = 90.71$, df = 3, $p < 0.001$), but also moderately significant for the other two (Cercaria 2 $X^2 = 10.07$ for 3 df, $p < 0.025$; and Cercaria 1 $X^2 = 4.22$ for 1df - the two higher and two lower sites pooled for $n > 5$ in each cell, $p < 0.05$). When percentage prevalences were plotted as a histogram (Figure 1.3) a gradient of decreasing infection from high to low water marks was clearly seen. A chi-square test for differences with maturity between the sites showed a similar trend. Figure 1.4 is a histogram showing percentages of adults and juveniles at each site. Adults were predominantly higher and juveniles lower on the shore ($X^2 = 31.23$ for 3 df, $p < 0.001$). Development of ovary in females was first apparent in snails between lengths 19 - 23mm; in males testis was observed in some individuals as small as 10mm length but was generally not apparent until 14 - 17mm. Fully mature gonad was found in all uninfected snails above these sizes.

	Total Pooled Data	%	Pooled 3-sample Data	%
Cercaria 1	113	5.77	31	5.31
Cercaria 2	252	12.86	98	16.78
Carcaria 3	177	9.04	84	14.38
Total Infected	(542)	(27.67)	(213)	(36.47)
Uninfected	1417	72.33	371	63.53
Total	1959	100.00	584	100.00

Table 1.1 - Prevalence of infection in *Cominella glandiformis* at the Avon-Heathcote estuary, alongside Rockinghorse Road. TOTAL POOLED DATA and POOLED 3-SAMPLE (January 1987, December 1987, February 1988) DATA.

	Site 1	%	Site 2	%	Site 3	%	Site 4	%	Total	%
rcaria 1	12	7.10	9	5.55	9	6.72	1	0.86	31	5.31
rcaria 2	32	18.93	26	16.04	22	16.42	18	15.52	98	16.78
rcaria 3	58	34.32	14	8.64	6	4.68	6	5.17	84	14.38
tal Infected	(102)	(60.36)	(49)	(30.25)	(34)	(25.38)	(25)	(21.55)	(213)	(36.47)
infected	67	39.64	113	69.75	100	74.62	91	78.54	371	63.53
tal	169	100.00	162	100.00	134	100.00	116	100.00	584	100.00

Table 1.2 - Prevalence of infection in *Cominella glandiformis* at four shore levels of the Avon-Heathcote estuary, along Rockinghorse Road - POOLED 3-SAMPLE (January 1987, December 1987, February 1988) DATA.

Site 1 = 0-20m from MHWS. Site 2 = 20-40m from MHWS.

Site 3 = 40-60m from MHWS. Site 4 = 60-80m from MHWS.

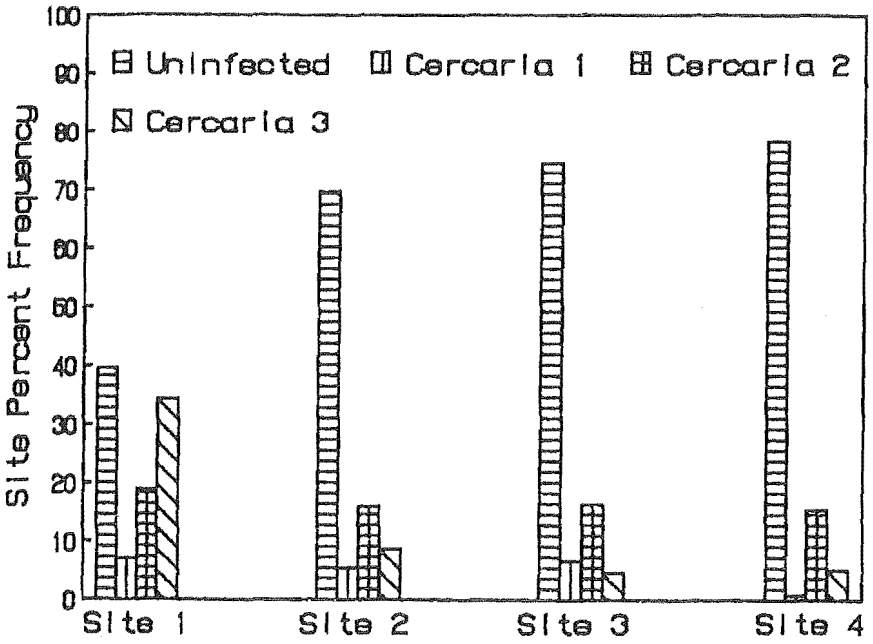


Figure 1.3 - Frequency histogram showing infection prevalences (%) in *Cominella glandiformis* at four different levels on the shore. Site 1 (0-20m from MHWS) represents the high tide area, with sites 2 (20-40m below MHWS), 3 (40-60m below MHWS), and 4 (60-80m from MHWS) representing a gradient towards lower tide areas.

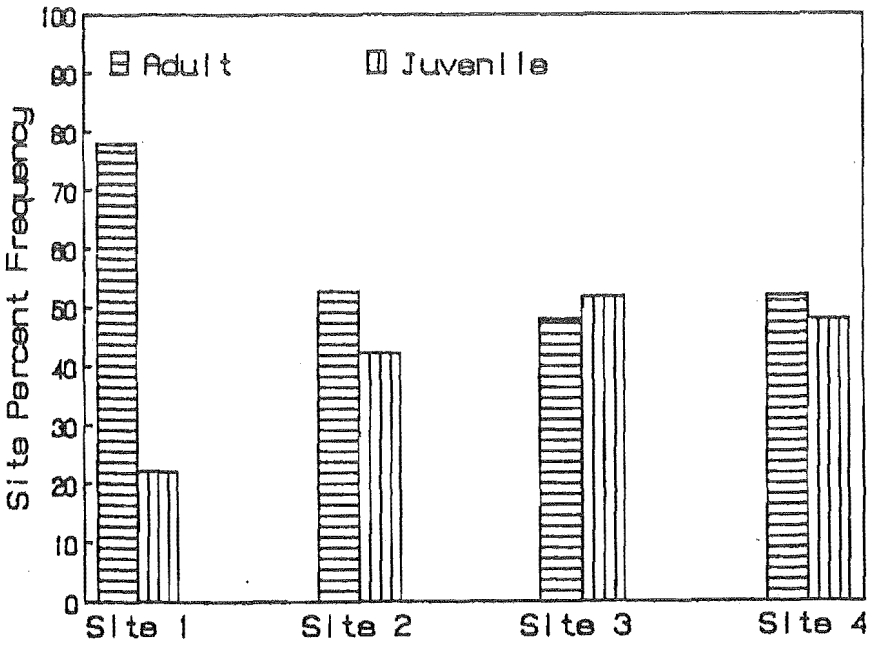


Figure 1.4 - Frequency histogram showing percentage prevalences of adult and juvenile *Cominella glandiformis* at four different levels on the shore. Site 1 (0-20m from MHWS) represents the high tide area, with sites 2 (20-40m below MHWS), 3 (40-60m below MHWS), and 4 (60-80m from MHWS) representing a gradient towards lower tide areas.

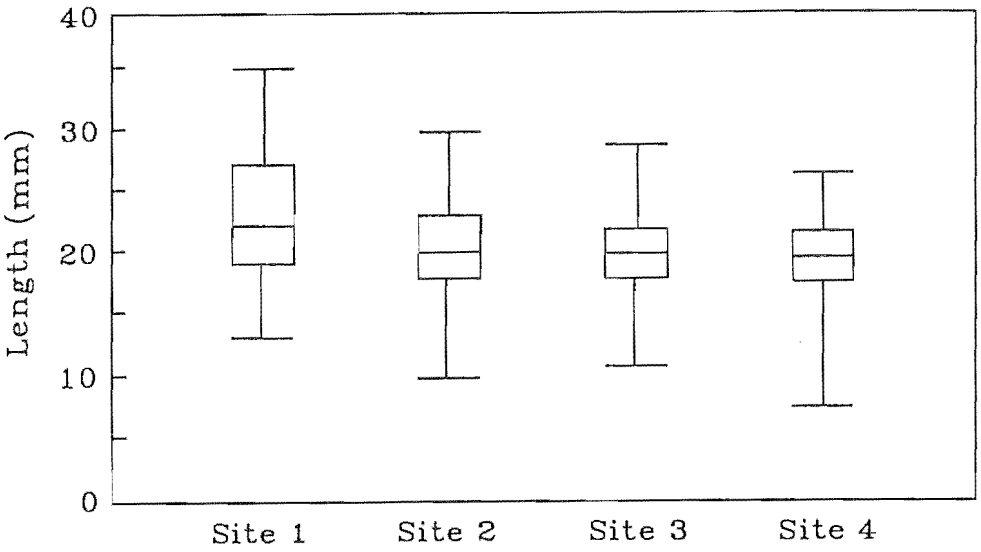


Figure 1.5 - Box chart plot showing the range of observed lengths for FEMALE *Cominella glandiformis* collected from four different levels on the shore. Site 1 (0-20m from MHWS) represents the high tide area, with sites 2 (20-40m below MHWS), 3 (40-60m below MHWS), and 4 (60-80m from MHWS) representing a gradient towards lower tide areas. Boxes are defined by the 25th and 75th percentiles, and enclose the median length value for each site (indicated by bar).

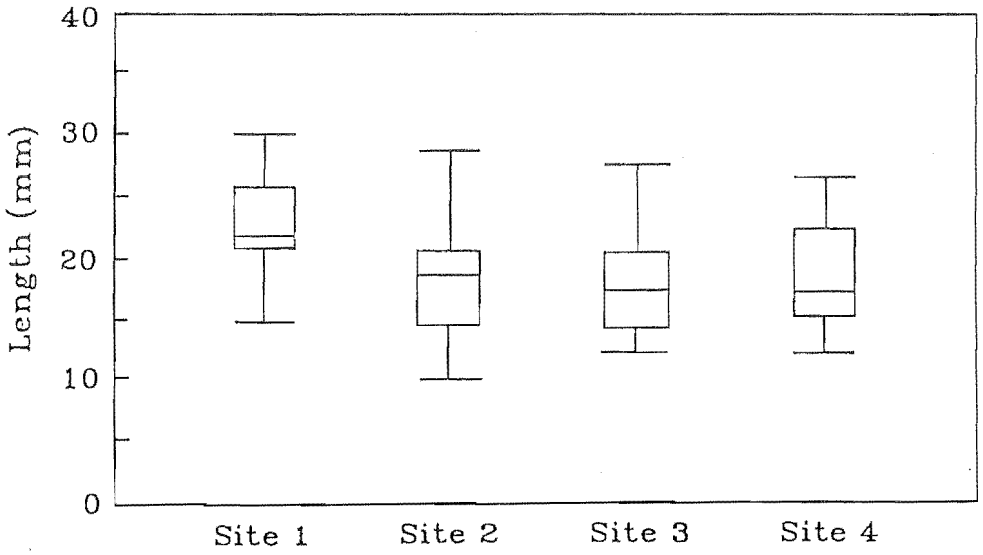


Figure 1.6 - Box chart plot showing the range of observed lengths for MALE *Cominella glandiformis* collected from four different levels on the shore. Site 1 (0-20m from MHWS) represents the high tide area, with sites 2 (20-40m below MHWS), 3 (40-60m below MHWS), and 4 (60-80m from MHWS) representing a gradient towards lower tide areas. Boxes are defined by the 25th and 75th percentiles, and enclose the median length value for each site (indicated by bar).

Box chart plots of length ranges for females and males are shown in Figures 1.5 and 1.6. Boxes are defined by the 25th and 75th percentiles, also enclosing the median length value for each site. For snails of both sexes, larger individuals were found higher on the shore. At site 1, in particular, both upper and lower range values exceeded those from other sites as did median length.

For stepwise discriminant analysis, data from nine of the samples were pooled for a total of 1748 individuals. Size data (length, tissue wet and dry weights, and shell weight) were log-transformed to meet the required assumption of equal within-group covariances. Canonical components, %SCV, and U-statistics for each discriminant analysis are shown in Table 1.3

Separate discriminant analyses with each infection type (Cercariae 1, 2, and 3) against non-infection all showed similar results. Cercaria 1 infections were discriminated on the basis of length only, a jack-knifed classification showing that 67.1% of cases could be correctly assigned to their original groups. Cercaria 2 infections were discriminated by both length and shell weight with 72.3% of cases correctly classified. The canonical variable for Cercaria 3 analysis also comprised length and shell weight, the jack-knifed matrix showing 74.5% correct classification. When data for the three infection types were run against each other (excluding non-infected), only 37.4% of cases in the jack-knifed matrix correctly classified indicating the groups could not be clearly distinguished. Shell weight was the only component of the canonical variable. Based on this, all infected data were pooled to one group.

Analysis of pooled infection versus non-infection resulted in a

canonical variable consisting of length and shell weight. The jack-knifed classification showed that 73.2% of the cases were correctly assigned. A histogram of the canonical variable for this

Analysis	Canonical Components	% SCV	U Statistic
Cercaria 1 vs Non-infected	Length	100.00	0.9081
Cercaria 2 vs Non-infected	Length	62.00	0.7879
	Shell weight	38.00	0.7844
Cercaria 3 vs Non-infected	Shell weight	51.28	0.7955
	Length	48.72	0.7984
Cercaria 1 vs Cercaria 2 vs Cercaria 3	Shell weight	100.00	0.9702
Pooled Infection vs Non-Infection	Length	62.48	0.7076
	Shell weight	37.52	0.7008

Table 1.3 - Canonical components, % contribution to the canonical variable (derived from standardised (by pooled within variances) coefficients), and U-statistics (Wilks' lambda) for individual stepwise discriminant analyses.

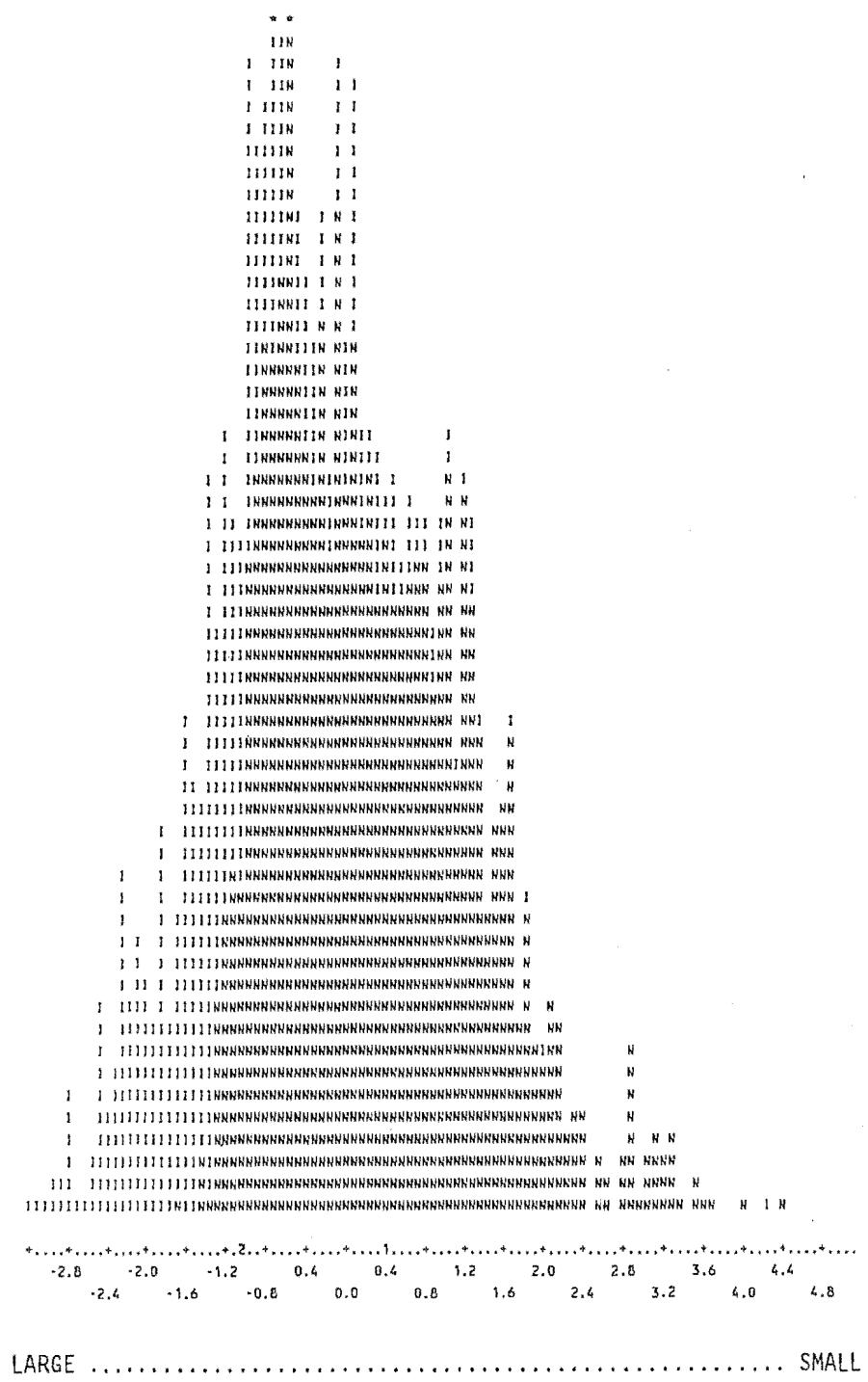


Figure 1.7 - BMDP generated histogram of the canonical variable showing the size distribution (based on the canonical variable) of infected (I) and non-infected (N) animals resulting from an analysis using pooled infection against non-infection.
The scale represents the standardised coefficient of the canonical variable derived from standardised values of length and weight.

analysis (Figure 1.7) showed an approximately normal distribution with infected individuals dominating the upper size (by canonical variable) classes.

Scatterplots and regression equations for size variables against each other (i.e length, tissue wet and dry weights, and shell weight) for total data (N = 1748) are included for future reference as appendix A. Correlation coefficients between the variables were all significant ($p < 0.001$), however that for length and shell weight ($r = 0.906$) was greatest. Although length and shell weight were the most effective discriminators in this study, either wet or dry tissue weights could therefore also be used. The closer the correlation between variables, the more likely it is that differences in a sample will result in shifts affecting which variables are considered the more important discriminators. Tissue wet and dry weights were not included in the discriminant function, therefore, probably because of sampling effects.

1748 individuals were also used for the MANOVA; infected data were pooled to one group. Maturity, sex, and infection were the between factors and given equal weighting. Overall the data could be significantly divided ($p < 0.0001$) by size (length, tissue wet and dry weights, and shell weight) into groups of either male/female, infection/non-infection, and adult/juvenile. Significant interactions ($p < 0.0001$) were also found between maturity and infection indicating that a split of the data (according to size) into adult/juvenile groups was the same as that for infected/non-infected groups (i.e. adult were associated with infection, and juveniles were associated with non-infection). Subsequent analysis with juvenile- and

adult-only data sets showed that within the juvenile group (728 cases) size variables overall could be significantly ($p < 0.01$) differentiated with respect to sex only. However a mildly significant difference ($p < 0.025$) was found between infected and non-infected juveniles with respect to length.

The adult group (1020 cases) gave more varied results. All dependent variables were significant with respect to both sex and infection ($p < 0.0001$), however a significant interaction ($p < 0.0001$) between the two made it unclear as to which was the greater source of variation.

Discussion.

Stepwise discriminant analysis of the data was a useful tool for establishing which of the variables recorded (length, Twet, Tdry, and shell weight) were most appropriate as an index of size. Approximately 70% of the data could be correctly classified into their original groups using some combination of length and shell weight in the canonical variable. Legendre and Legendre (1983), from other studies, alluded to a similar figure as acceptable for such classification. However when one examines the U-statistic associated with these classifications it is apparent that the majority of variance remained unaccounted for. For the overall classification (total pooled infection vs non-infection, $U = .7008$) some seventy percent of the variance was unexplained. Therefore, although the data could be reasonably discriminated on the basis of size (length, and

shell weight) I feel that the groups tested (infected and non-infected) were not truly separated. This is further evidenced, in the histogram of the canonical variable, by a large degree of overlap between the two groups with only one peak for an approximately normal distribution. Were the groups truly separate two distinct peaks should be apparent.

Analysis overall did show that prevalence of all three parasites increased with snail size. Ryburn (1972), in an earlier study on infection in *Cominella glandiformis*, reported similar positive correlations with size for Cercaria 2 but negative correlations with Cercaria 3 infection. The discrepancy between these studies probably arose from Ryburn having sampled mainly from low tide areas (site 4 in my samples) where both larger snails (above 27mm length) and the majority of Cercaria 3 infections are rare. Largest individuals of both sexes were found higher on the shore, as were adults. This is not surprising as MANOVA indicated that maturity and infection grouped similarly with respect to size. Juveniles predominated in areas at least 20m below MHWS.

Irrespective of maturity, MANOVA also showed that size differences exist between sexes. The length at which most females were sexually mature was greater than for males, and females had a wider size range than males. Sousa (1983) cited evidence showing that it is common among prosobranch molluscs for pre-reproductive females to grow at a faster rate than males. Indeed, as the fecundity of females increases with size (Hughes and Answer 1982), those growing to a relatively large size at first reproduction are at a selective advantage. Hoagland (1978) (cited Sousa 1983) suggested that smaller males are more mobile with a better chance of fertilising females than larger,

more sedentary, individuals.

In any study, such as this, where infection prevalence is found to increase as snails become larger, the possibility that gigantism may be the cause of such correlations must always be acknowledged. From the analyses described, both infected and non-infected snails appeared to fall into one population, the uppermost size ranges of which were coincidentally infected by larval flukes. Based on distribution on the shore infection would appear to accrue in the largest snails simply because they are older with the most chronic exposure to miracidia. As individuals age they probably also move higher on the shore into areas of greater exposure to miracidia, further compounding the probability of infection (Cannon (1979) found this in a similar study). These results, however, do not preclude gigantism. The effect may be such as to increase the average size range of infected snails beyond that of non-infected, while still keeping within the range of atypical non-infected individuals growing naturally to a size above average. Final conclusions as to cause of the correlation effect can only be formed from a laboratory study following growth rates of both infected and non-infected individuals (preferably from the same initial size/age), or at the very least from a capture-recapture study with similar aims.

CHAPTER 2.

GENERAL HISTOLOGY, AND ANTHELMINTIC ELIMINATION OF PARASITE INFECTION IN *COMINELLA GLANDIFORMIS*.

Introduction.

All three digeneans infecting *Cominella glandiformis* at the Avon-Heathcote estuary appear to follow the generalised pattern of flukes within snail hosts. Once miracidial penetration occurs, infection normally localises in the digestive gland (= hepatopancreas) and gonad regions.

The extent of damage incurred by a host varies not only between species of parasite but also within any one type of infection. One way of distinguishing between the destructive potential of larval flukes may lie with structural differences between rediae and sporocysts (stages of the parasite life-cycle responsible for asexual reproduction within the snail). Sousa (1983) considered rediae more destructive as they posess a pharynx enabling active feeding. Rediae also possess procuscula (ventral lobe-like processes) which may be used for leverage against host tissue allowing greater movement (Rees 1966; Erasmus 1972). Sporocysts, on the other hand, are incapable either of ingesting tissue, or of great movement, and tend to rely instead on absorption of nutrients across the body wall.

Host snail castration is generally considered a normal consequence of larval fluke infection. The form of castration varies, however, and both the extent and means will affect the final outcome of the interaction. Minchella (1985), for example, suggested that if

reproduction was completely inhibited then,

"long-lived (perennial) snail hosts may respond by utilising energy normally used for their own or the trematodes reproduction for enhanced growth ... in order to compromise parasite reproduction and to improve host survival in hopes of outlasting the parasitic infection and its negative effects on reproduction."

For such an adaptation to occur the negative effects of infection must be short-lived relative to the maximum life of the snail, which must then itself be capable of recovering to reproduce. Presumably, if host reproduction remains possible, to any extent, whilst infected it is more advantageous for the snail to gain immediate reproductive success and use its resources for gamete formation instead of giant growth.

Clearly, details of gonad pathology, the form and extent of castration, and some knowledge of host recuperative abilities are required in order to correctly interpret any snail/parasite interaction (particularly if growth phenomena are associated with infection in the field or laboratory). To these ends a study of both general histology, and the recovery of *Cominella glandiformis* upon parasite elimination, was undertaken.

Methods.

1. General Histology.

Snails were hand collected from the field study sampling area (see previous chapter) of the Avon-Heathcote estuary. Shells were gently cracked by hammer and the soft body parts removed as intact as

possible for inspection under the dissecting microscope. Gonad and digestive glands of forty snails - including ten typical of each of the three infection types and ten non-infected - were fixed in Bouin's solution for 24 hours. After this the yellow fixative was leached by successive changes of 70% ethanol (saturated with LiCl) until no colour remained. The tissue was further dehydrated through changes of 90% and 100% ethanol, then cleared in terpineol for 24 hours. Following this the tissue was embedded in paraffin wax under vacuum (3 changes) and sectioned by microtome to 7μm. Sections were stained with Ehrlich's haematoxylin and eosin, mounted in Eukitt, and finally examined under the compound microscope.

2. Elimination study.

All snails were hand-collected from the same sampling area of the Avon-Heathcote estuary as the field study. These were maintained in perforated 2-litre plastic containers (up to twenty snails each) within laboratory marine aquaria and fed 2 to 4 (depending on size) cockles (*Austrovenus stutchburyi*) each week for the study duration.

Trials were set up attempting to eliminate infection through the use of anthelmintics. Moser et al. (1986) successfully used praziquantel in aquarium water to rid freshwater snails of their parasite loads, however their efforts involving marine snails were not successful. It was suggested that injection of the drug may be more effective in marine snails. My initial trials on *Cominella glandiformis* involved injecting either 0.005mg or 0.01mg praziquantel (available as Droncit 50mg tablets, ground to powder and diluted with physiological saline) into the foot of each snail. These dosages were comparable to the quantity of drug available to freshwater snails in

Moser's study, but resulted in little success. I therefore decided to use higher doses of this drug and also to try two other anthelmintics - Levamisole and Ivermectin, available in liquid form of respective stock strengths 40mg/ml and 10mg/ml, diluted to final concentrations with physiological saline.

Fifteen to twenty snails, all larger than 22mm length (ensuring adults only) were used in each dosage/drug trial. Individuals were not screened for parasites before the experiments, however field data suggested that there was a high probability of infection in the size range used. Any non-infected individuals would serve as controls for the infected group. Drugs were injected into the foot of individual snails at one of the following dosages - Ivermectin 0.05mg, 0.10mg, 0.15mg, 0.20mg, and 0.25mg; Levamisole 0.10mg, 0.20mg, 0.25mg, and 0.30mg; Praziquantel 0.10mg. Another fifteen individuals were offered cockles each injected with 1.0mg praziquantel to see if the drug was more effective when ingested with food.

Study periods varied depending on the reaction by snails to each drug and dosage but typically did not exceed four weeks. Histological examination was made of all snails in which infection appeared different, or less active, by comparison with either field collected animals or untreated controls held in aquaria for a similar time period.

Results.

1. General Histology.

a. Gross appearance of tissue.

When examining dissected tissue under the stereo microscope each infection could be clearly differentiated upon examination of the dorsal surface of the digestive gland. Rediae of Cercaria 1 were immediately visible as large white sausage shaped sacs capable of much movement within host tissue. Large (approx. 1mm length) mature cercariae were frequently observed wildly thrashing about having left rediae via the birth pore. Of the three fluke species, light infections were found only for Cercaria 1 but these totally occupied gonad tissue while leaving the digestive gland virtually intact. Cercaria 2 infections manifested as a translucent orange colour over the entire digestive gland/gonad region. This was due to the presence of small ball-like sporocysts containing numerous pigment granules. Tiny cercariae - approx. 0.18mm length, visible only at higher magnifications - usually swam en masse from the sporocysts upon dissection. Although Cercaria 3 infections typically consisted of orange sausage-shaped sporocysts, a large number of these (sometimes up to to 60%) were instead black/brown over the entire sporocyst surface. Dissection of sporocysts revealed the tailless cercariae, approximately 0.8mm length, incapable of much movement other than crawling.

b. Histological examination of snail hepatopancreas and gonad.

Uninfected tissue appeared different depending on sex and maturity

of the snail. In juvenile snails of either sex, gonad had yet to develop, and only hepatopancreas tissue was evident. Photo 2.1 is a section of uninfected juvenile male digestive gland showing clearly the numerous tubules, composed of digestive and secretory cells (James 1965), apparent throughout the hepatopancreas. The cells surround the tubule lumen into which food is passed from the stomach. Such food particles are ingested by the ciliated border of the cells, and enclosed in food vacuoles (James 1965).

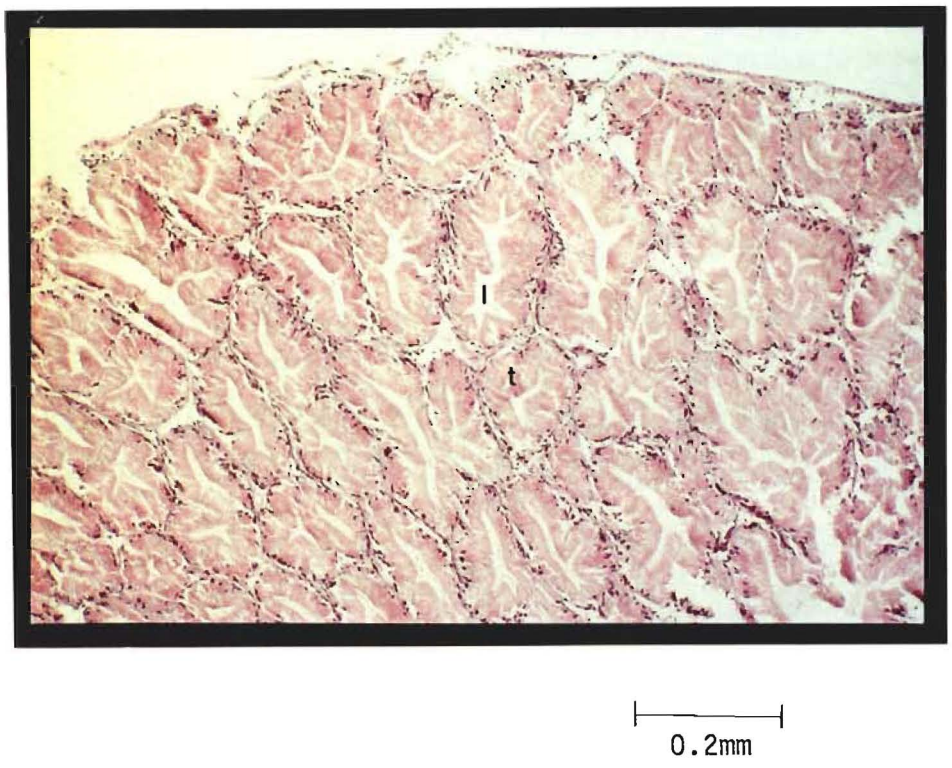


Photo 2.1 - Section through the digestive gland region of an uninfected juvenile snail. The digestive gland consists of numerous tubules (*t*) which surround the tubule lumen (*lu*).

In adults, a large proportion of the dorsal surface of digestive gland lay beneath gonad. In females the ovary was usually granular and bright orange, covering one third of the hepatopancreas. Sections showed this to contain numerous developing ova surrounded by a mass of yolk cells (Photo 2.2). Testis in male snails was a translucent yellow/cream and covered slightly more area. Sections through testicular tissue showed large numbers of testicular tubules, each with large concentrations of spermatogonia, and thread-like spermatozoa (Photo 2.3). Very little detail could be distinguished other than this.

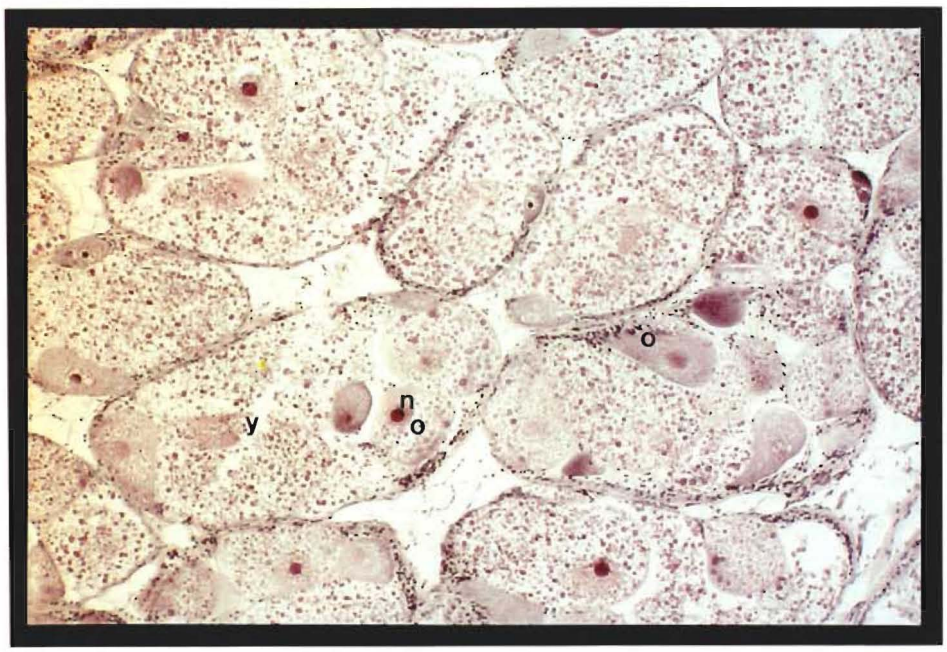


Photo 2.2 - Section through the gonad region of an uninfected adult female snail showing ova (*o*) at varying stages of development, surrounded by yolk masses (*y*). The nucleus (*n*) of each ovum is deeply stained.

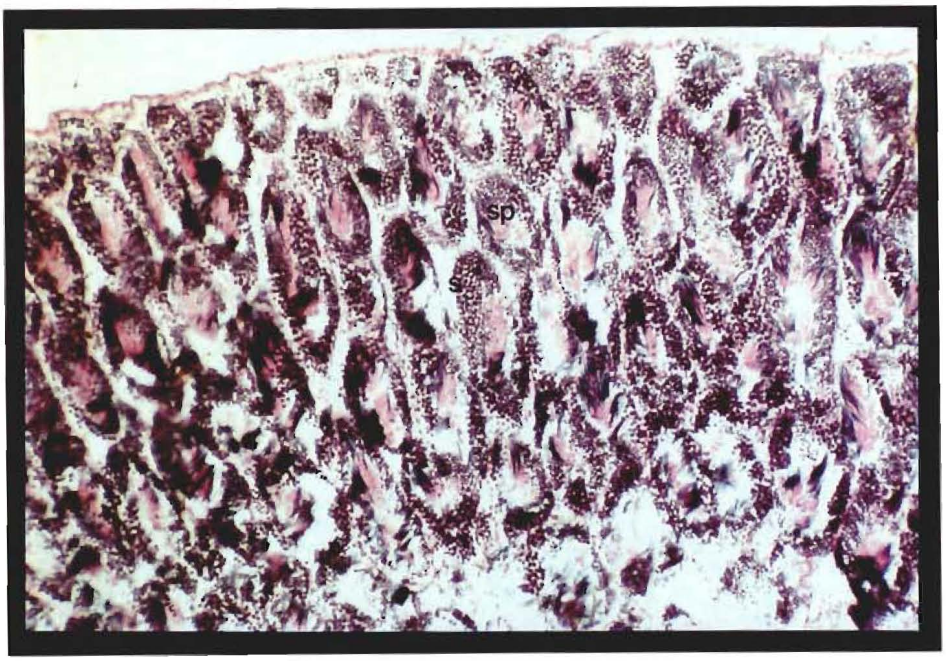


Photo 2.3 - Section through the gonad region of an uninfected adult male snail showing testicular tubules, spermatogonia (*s*) and spermatozoa (*sp*).

Photos 2.4, 2.5, and 2.6 show sections of Cercariae 1, 2, and 3 infections respectively. Developing cercariae are clearly seen within the rediae and sporocysts, which themselves occupy most of the intertubular zones. This was typical of both digestive gland and gonadal regions. Gonad tissue typically atrophied to the point where sections through this region were virtually indistinguishable from those through the hepatopancreas. The lumen of both digestive gland and gonadal tubules were compressed by all three types of infection and probably damaged to the point of non-function. Generally, in any

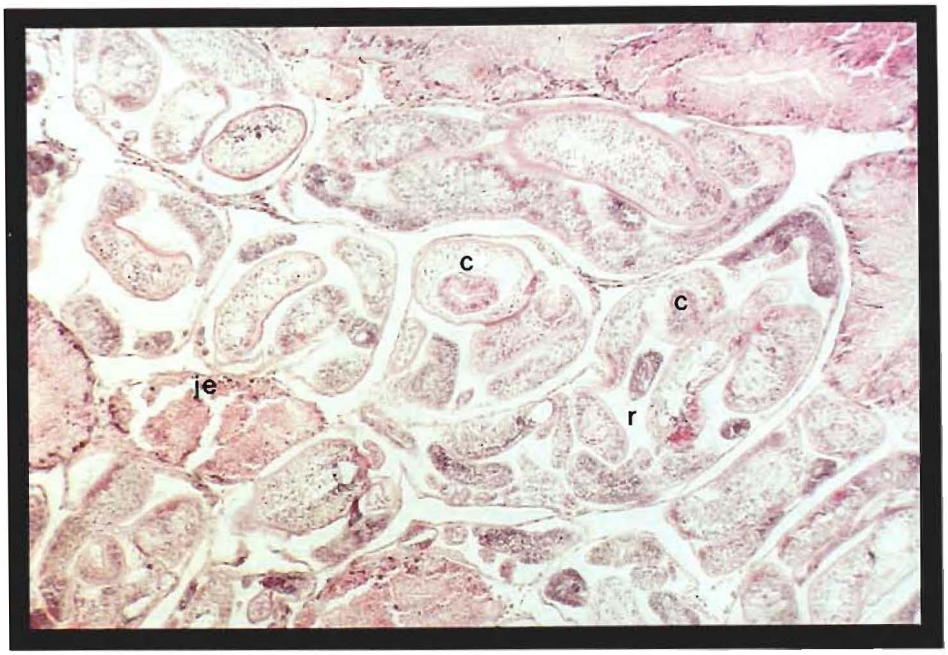


Photo 2.4 - Section through digestive gland region of a snail with a CERCARIA 1 infection. Cercariae (*c*) lie within the rediae (*r*). Jagged edges (*j.e.*) indicate mecahnical damage, due to infection, of digestive gland cells.

infection, pressure from growing parasite germinal sacs can crush the digestive gland cells and close the lumen preventing any food from passing from the stomach to the digestive cells (James 1965). Compression by the parasite leading to cell rupture and mechanical damage is usually typified by jagged edges along the cells (James 1965), seen in Cercaria 1 infections (Photo 2.4). Such mechanical destruction was not evident, however, in the other two infections.

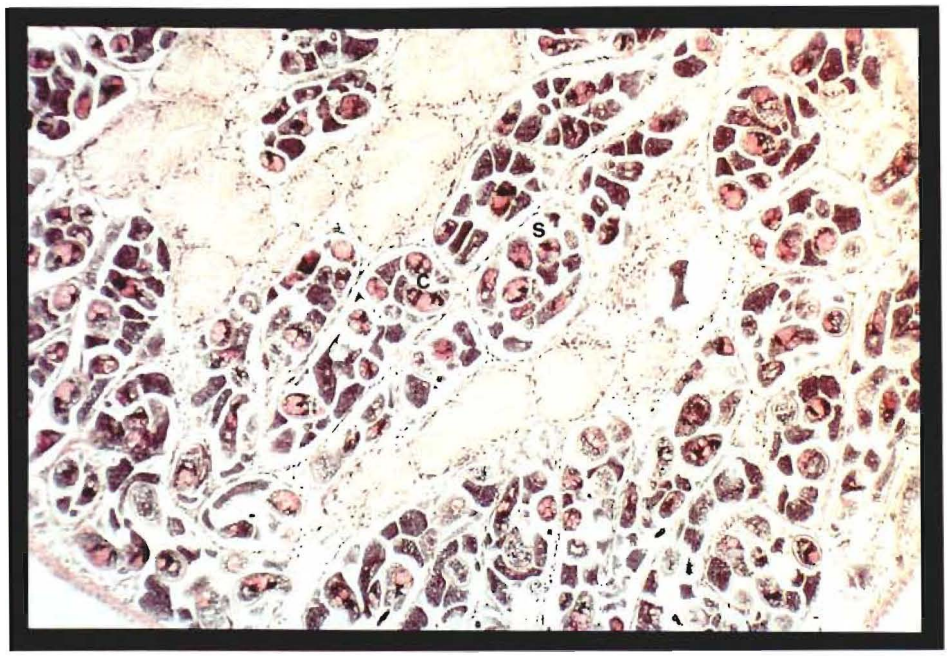


Photo 2.5 - Section through digestive gland region of a snail with a CERCARIA 2 infection. Numerous small cercaria (*c*) are seen within each sporocyst (*s*).

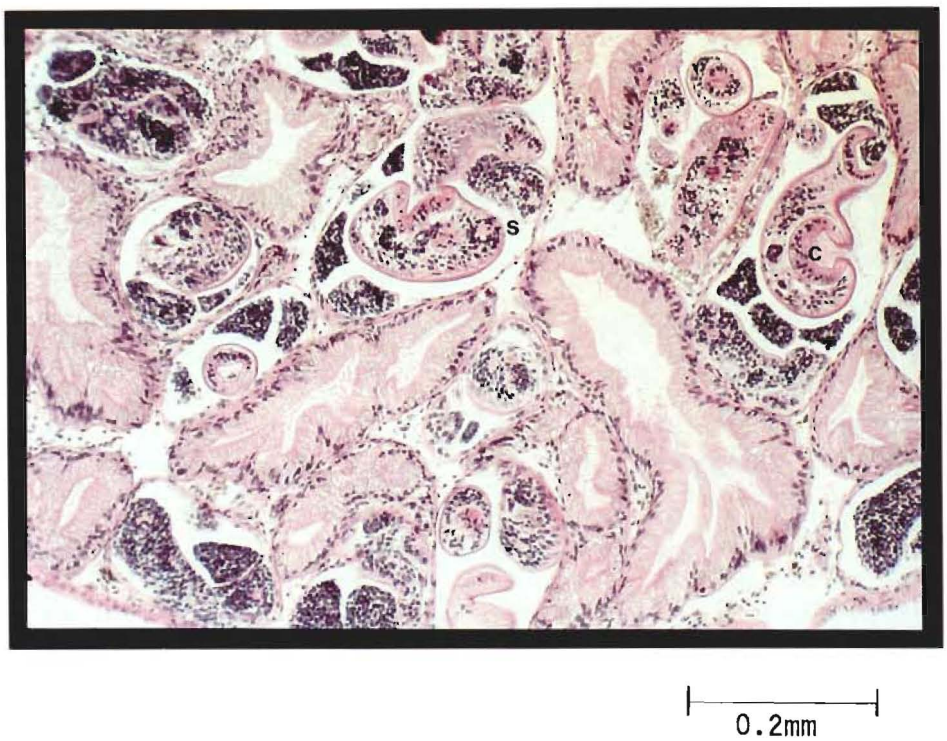


Photo 2.6 - Section through digestive gland region of a snail with a CERCARIA 3 infection. Cercariae (*c*) are clearly seen within each sporocyst (*s*).

Malek and Cheng (1974) described parasitic castration as resulting from two processes - mechanical and physiological. No evidence was found however, either of ingested material within rediae, or any form of mechanical destruction other than just described. Gonad tissue was found in only four of the infected snails examined. All four were female and comprised one Cercaria 1 and three Cercaria 2 infections. In each case yolk cells, comparable in size and stucture to those of uninfected animals, were interspersed with redia or sporocysts in the gonadal region. Apparently mature ova were also present with such yolk in the three Cercaria 2 infections (Photo 2.7). A comparison of

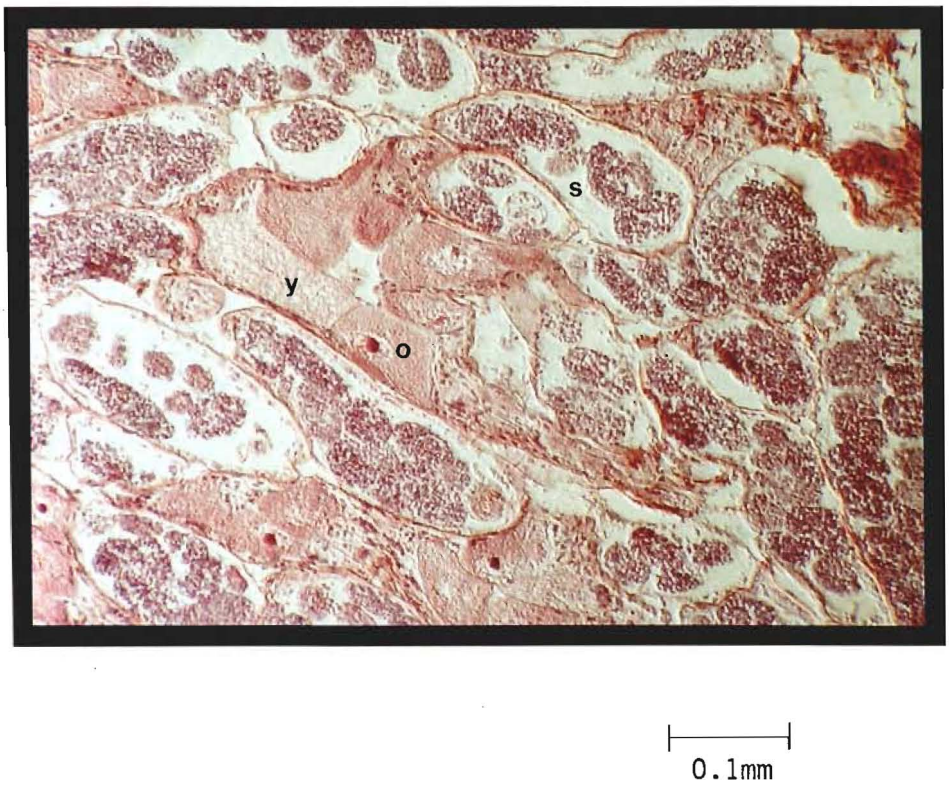


Photo 2.7 - Section through the gonad region of a snail with a Cercaria 2 infection showing apparently mature ova (*o*) and yolk (*y*) amidst parasite sporocysts (*s*).

ova from parasitised snails with those from uninfected showed both were of approximately similar sizes. The average length of uninfected ova was 0.11 mm with a width of 0.06mm (N = 12), whilst those from infected sections (N = 6) averaged 0.10 mm length and 0.06 mm width. Where such ova were evident alongside infection, the respective infections were well established and would have been patent for some time before collection of the host. The presence of ova therefore more likely indicates *bona fide* gametogenesis concurrent with infection rather than residual ovary yet to deteriorate.

Photo 2.8 - Section through digestive gland region of a snail showing the brownish-yellow (non-staining) spores (*s*) of *Urosporidium* sp. typically associated with Cercaria 3 infections. Many of the surrounding redia (*r*) appear to have deteriorated.

Sections through the black/brown sporocysts in Cercaria 3 infections showed them to contain a large number of brown ball-like masses (Photo 2.8). In many cases fibroblastic granulomas - a typical molluscan immune response to parasitic invasion (Pan 1965; Malek and Cheng 1974) - had formed around the masses which were trapped within remaining scar tissue (Photo 2.9). The "balls" were subsequently identified as spores of *Urosporidium* sp., a hyperparasite of larval digeneans. A brief description of this is given as the following chapter.

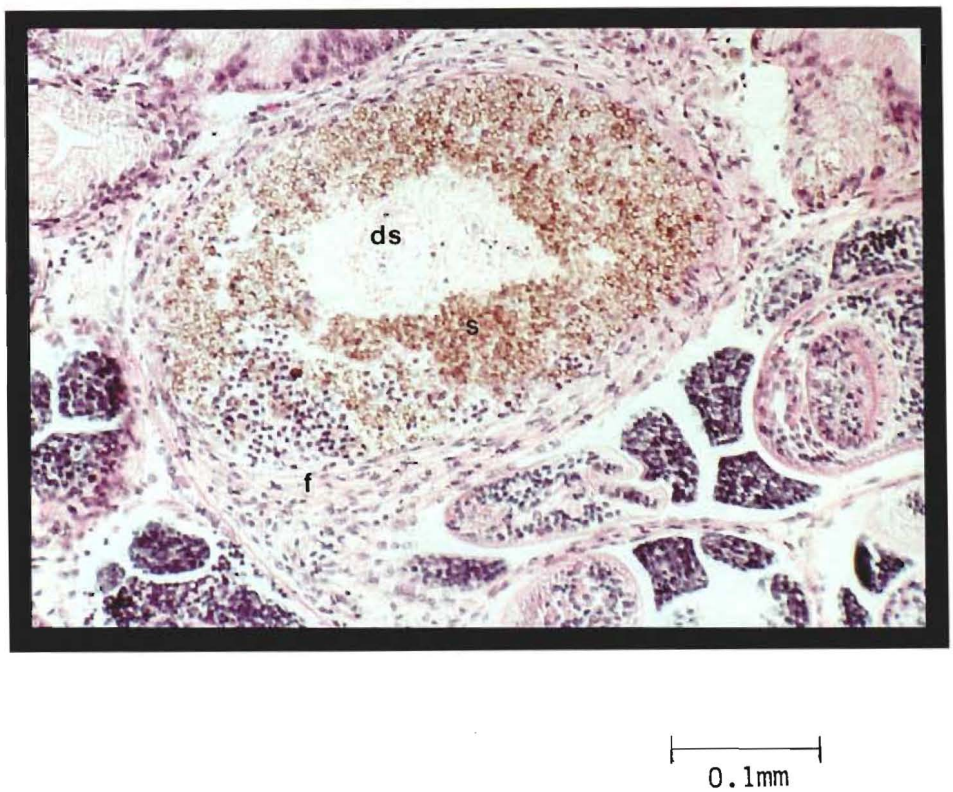


Photo 2.9 - Section showing a granuloma associated with hyperparasitic infection of Cercaria 3 rediae by *Urosporidium* sp. Fibroblasts (*f*) form the granuloma matrix that surrounds mature spores (*s*) which have accumulated within a deteriorated sporocyst (*d.s*).

2. Elimination study.

a. *Praziquantel*. All twenty snails which were injected with 0.10mg praziquantel were dead or dying within four days. This was not unexpected as earlier trials at lower drug concentrations also resulted in high rates of mortality. Five of eight snails surviving to the fifth day post- treatment harboured infections - three Cercaria 2, and two Cercaria 3 - however these appeared unaffected and normal.

Snails (N = 17) fed on praziquantel-injected cockles were maintained for five weeks in the laboratory. During this time, however, very little feeding on the treated cockles was recorded, whereas occasional supplements of untreated food were completely consumed. Seven infections were present within the group - four Cercaria 3, two Cercaria 2, and one Cercaria 1 - but all appeared active. The snails may have detected the presence of the drug and avoided feeding resulting in little exposure to the anthelmintic.

b. *Ivermectin*. A summary of snail numbers surviving each treatment, their condition, the types of infection found, and the appearance of each infection are given in Table 2.1. Infections listed as inactive were healthy in appearance but with little cercarial activity, whereas those classed as abormal appeared neither healthy or active but still comprised live sporocysts. No dead infections were found under treatment with this drug, however a high snail mortality was apparent, especially at higher concentrations (0.20mg and 0.25mg).

DOSAGE (mg)	SURVIVORS & TIME PERIOD	INFECTION TYPE	SNAIL CONDITION		INFECTION STATUS	
0.05	14/15	9 x Uninfected	Normal		Nil	
	4 weeks	3 x Cercaria 2	Normal	...	Normal	
		2 x Cercaria 3	Normal	...	Normal	
0.10	13/15	2 x Uninfected	Normal		Nil	
	4 weeks	8 x Cercaria 2	Normal	...	1 Inactive	*
				...	7 Normal	
		3 x Cercaria 3	Normal	...	Normal	
0.15	6/15	3 x Uninfected	2 Normal, 1 Dying		Nil	
	1 week	2 x Cercaria 2	Normal	...	1 Abnormal	*
				...	1 Inactive	*
		1 x Cercaria 3	Normal	...	Abnormal	*
0.20	4/15	3 x Uninfected	Dying		Nil	
	1 week	1 x Cercaria 2	Dying	...	Inactive	*
0.25	4/20	3 x Uninfected	Dying		Nil	
	4 days	1 x Cercaria 2	Dying	...	Inactive	*

Table 2.1 - Summary results table of treatment with Ivermectin.
Asterisk (*) denotes those snails examined histologically.

Histological examination of sections through the gonad-hepatopancreas regions of six apparently abnormal or inactive infections did not differ from those of non-treated individuals (see section 1, this chapter). Little evidence was found suggesting that Ivermectin is an effective anthelmintic in this situation. Low concentrations of the drug had little effect whereas higher concentrations, although having some inhibitory action, did not eliminate infection before also killing the host. The high mortality rate could in part, however, have been due to the formation of a slight precipitate when stock drug (10 mg/ml) was diluted to lower concentrations.

c. *Levamisole*. Table 2.2 is a summary of the results from each dosage trial with Levamisole. One dead Cercaria 1 infection was found in a snail given the highest treatment - 0.30mg Levamisole. Histology on this tissue revealed shrunken redial walls and some deterioration of the cercariae contained within, however the digestive gland and gonadal tubules remained in a damaged state with no gonad (testis, ova, or yolk) evident. Two other Cercaria 1 infections at this dosage were inactive and some ovary was also present, mainly in the form of yolk cells. No mature or developing ova were evident.

Only the highest concentrations of Levamisole given experimental snails had any observed effect on infection, however even these were not completely effective. Greater concentrations of the drug are probably required to fully rid snails of their parasite loads. Fortunately the snail mortality rates accompanying treatment with this drug were low enough to suggest that a satisfactory higher dosage could be achieved without endangering too many host individuals.

DOSAGE (mg)	SURVIVORS & TIME PERIOD	INFECTION TYPE	SNAIL CONDITION		INFECTION STATUS	
0.10	10/15	6 x Uninfected	Normal		Nil	
	2 weeks	2 x Cercaria 2	Normal	...	Normal	
		2 x Cercaria 3	Normal	...	Normal	
0.20	15/15	11 x Uninfected	Normal		Nil	
	4 weeks	1 x Cercaria 2	Normal	...	Normal	
		3 x Cercaria 3	Normal	...	Normal	
0.25	13/15	9 x Uninfected	Normal		Nil	
	3 weeks	1 x Cercaria 1	Normal	...	Normal	
		3 x Cercaria 2	Normal	...	2 Normal	
				...	1 Inactive	*
0.30	14/15	9 x Uninfected	Normal		Nil	
	4 weeks	3 x Cercaria 1	Normal	...	1 Dead	*
				...	2 Inactive	$*
		2 x Cercaria 3	Normal	...	Normal	

Table 2.2 - Summary results table of treatment with Levamisole. Dollar ($) denotes some gonad concurrent with infection, asterisk (*) denotes those snails examined histologically.

Discussion.

The effects produced by parasites on the digestive gland and gonad of their hosts are probably both mechanical and physiological. The means of mechanical disruption were described earlier, but this was only obvious in Cercaria 1 (redial) infections. There can be no doubt, however, that equivalent damage was effected by the other two parasite species, probably by physiological means. All three parasite species probably employed chemical means of host modification to some extent. Proof of host hormonal interruption by digeneans has been demonstrated both for freshwater snails (Joose and Van Elk 1986) and marine (Pearson and Cheng 1985), but the parasite substances responsible were not isolated. Typically their action was to interfere either with the production of host secretions (e.g. gonadotrophins) and the effect of such hormones on their target organs (by inhibiting sensitivity to said hormones), or to directly lyse host tissue.

An early example of both mechanical and physiological damage to a host was reported by Rees (1936) in the winkle *Littorina littorea*, infected by both redial and sporocyst species. Redial damage was by ingestion and tissue atrophy due both to parasite pressure and waste production; damage by sporocysts was due to waste accumulation and pressure atrophy leading to starvation. Generally the effect on digestive gland cells by parasite compression is similar to conditions within a starving animal. James (1965) found that even when cells were not already killed or undergoing autoloysis from mechanical disruption, physiological effects such as decreases in food storage globules, and increases in acid and alkaline phosphatases and carbohydrate metabolism were always evident in response to parasite

excretory products in the haemocoel.

In the majority of snails castration was complete, however exceptions did occur where intact yolk cells and/or mature ova were present in parasitised animals. Rees (1936) and Cheng and Burton (1965) both reported similar phenomena but found the ova to be deteriorating. In both studies the ovary was blocked from food sources and thus developing oocytes failed to attain full size. From size comparisons with non-infected animals the ova found in *C. glandiformis* had not deteriorated but given overall condition of the gonad it seems unlikely any external deposition of mature eggs could occur.

A difference was apparent between Cercaria 3 infections and the other two digenean parasite species due to presence of the hyperparasite (*Urosporidium* sp.). No unparasitised Cercaria 3 infections were found although the extent of hyperparasitism varied. Wherever an infected sporocyst was present, some form of host immune response was also always evident. Lackie (1980) described phagocytosis and encapsulation as universal phenomena in invertebrates but under normal conditions most parasites are able to evade a host response either through non-recognition (e.g. inherent antigenic similarity to the host, or incorporation of host molecules onto their own body surfaces) or interruption of host effector mechanisms. Infection with *Urosporidium* sp., by ultimately killing the host sporocyst, upsets said evasion of snail immune systems invariably resulting in encapsulation of deteriorating sporocysts within granulomata and scar tissue. Such activity by the immune system would undoubtedly utilise additional host resources that might otherwise have been available for either host or parasite growth.

According to the hypothesis of Minchella (1985), earlier mentioned, host snails may utilise a strategy of "temporal-compensation" (Minchella et al. 1985) increasing their growth to allow greater chances both of limiting internal tissue damage and outliving the parasite to reproduce at a later date. Assumptions were i. that reproduction must be completely inhibited but castration indirect, and ii. infection must be short-lived with gonad regeneration possible. Another hypothesis - "energy allocation" (Minchella et al. 1985) - based on energy budgets, assumes that host post-infective growth is regulated by nutritional demands of the parasite (e.g. Sousa 1983). Although no ingested tissue was demonstrated within rediae of Cercaria 1 this probably occurs, however other direct physical damage was noted in the form of mechanical disruption to both hepatopancreas and gonad. A number of authors (e.g. Erasmus 1972; Malek and Cheng 1974) generally agree that hepatopancreas is the principal site of localisation by larval flukes. Cercaria 1 infections, however, primarily invaded gonad and used the resources therein before spreading to hepatopancreas secondarily. In such infection a high degree of damage to the gonad would seem inevitable. Consequently, the assumptions for temporal-compensation are not met for this species of infection.

Some Cercaria 2 (sporocyst) infections contained apparently mature ova indicating that castration was not complete in all cases. One should not, however, equate reproductive success with gamete formation - due to parasite disruption the likelihood of viable eggs being fertilised and/or deposited is such that reproduction would still appear effectively inhibited. If, as Rees (1936) and Cheng and Burton (1965) suggest, such ova were likely to deteriorate then Minchella's

assumptions could in any case be met as direct tissue destruction was not noted. Gigantism through temporal-compensation could therefore be considered a viable strategy for such snails.

Cercaria 3 infected snails might make good candidates for temporal-compensation but for the hyperparasitism. Although castration appeared both complete and indirect, energy requirements for the additional burdens of massive granuloma and scar tissue formation would probably preclude the snail's use of its resources for either increased growth or gonad regeneration.

Little can be said as to host recovery from infection. Treatment with Praziquantel and Ivermectin was not successful - near-death conditions of the snail hosts treated with 0.20mg and 0.25mg Ivermectin could as easily have accounted for inactivity of the Cercaria 2 infections present as could the drug itself. Different (more soluble) forms of these drugs may be more suitable. Results of the Levamisole trials were encouraging and suggested that further work, either with still higher drug concentrations or repeated injections of concentrations already used, will succeed in eliminating infection. Host recovery mechansims can then be studied. However, even if host snails can recuperate (and resume reproduction) upon parasite elimination, this must also be demonstrated naturally in the field to validate the "temporal-compensation" hypothesis.

In conclusion, the overall effects of each fluke parasite species on host tissue appear quite similar. Differences between redial and sporocyst modes of activity are minor; both nutritional requirements and ultimate localisation within hepatopancreas and gonad appear the same.

CHAPTER 3.

A BRIEF DESCRIPTION OF *UROSPORIDIUM* SP., HYPERPARASITE OF A TREMATODE FROM *COMINELLA GLANDIFORMIS*.

Introduction.

During the course of my study on *Cominella glandiformis*, a haplosporidian protozoan - *Urosporidium* sp. - was found to infect one of three digenean parasites (Cercaria 3) also under investigation (see chapter 2). Hyperparasitised sporocysts were found in all snails carrying Cercaria 3 infections. Infected sporocysts ranged in number

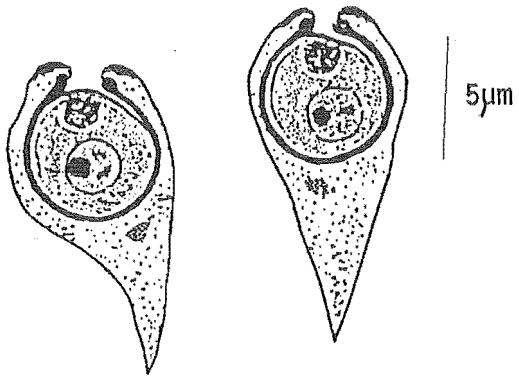


Figure 3.1 - Diagrammatical representation of mature spores of *Urosporidium* sp. hyperparasitising Cercaria 3 sporocysts from *Cominella glandiformis*.

from approximately 10% to 60%. Ormieres et al. (1973) listed only six described species belonging to this genus, one of which, *Urosporidium*

constantae, infects *Bucephalus longicornutus* sporocysts found in *Ostrea lutaria*, the New Zealand mud-oyster (Howell 1967). Five of the six species described are hyperparasites of either sporocyst or metacercarial digenean life-history stages. *Urosporidium* sp. are characterised by spores having an anterior orifice without an operculum and a long trailing tail formed of extraspore material (Perkins 1971) (Figure 3.1). Typically, infected sporocysts or metacercariae are most readily distinguished under the dissecting microscope by their large size and dark pigmentation (Howell 1967; Ormieres et al. 1973; Couch 1974).

Methods.

Study of *Urosporidium* infected sporocysts was by two means: light microscope and transmission electron microscope (T.E.M).

i. Light microscope - methods were identical to those for general histology on infected and non-infected *Cominella glandiformis*, chapter 2 (see pp 30-31). Measurements were taken by ocular micrometer and comprised at least ten individual spores or development-stages.

ii. T.E.M.- infected sporocysts were dissected from *C. glandiformis* under the dissecting miscroscope and fixed for 8 hours in 2.5% glutaraldehyde buffered with 0.1M sodium cacodylate (ph = 7.4). Tissue was rinsed in buffer for 24 hours at 4 °C, post-fixed in 1% osmium tetroxide for 4 hours, and again rinsed in buffer as before. Sporocysts were then dehydrated through an ethanol series to 100%, transferred to acetone for fifteen minutes, and embedded in Spurr's (soft) epoxy resin via an acetone-resin series which was cured at 70°C

for 24 hours. Embedded tissue was cut on an LKB 8800 Ultrotome III to produce thin sections which were double-stained with saturated uranyl acetate in 70% ethanol, and 0.5% lead citrate. The sections were viewed under a JEOL T.E.M. - JEM 1200-EX - at an accelerating voltage of 80 Kv.

Results.

Under the light microscope, spores were readily apparent as unstained brownish-yellow "balls" with trailing tails (Photo 3.1).

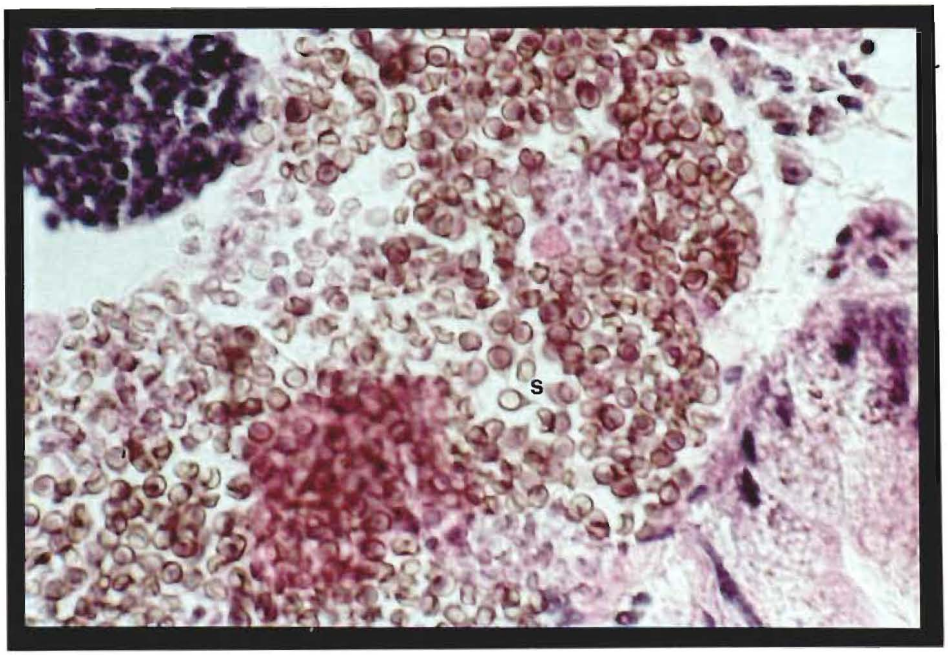
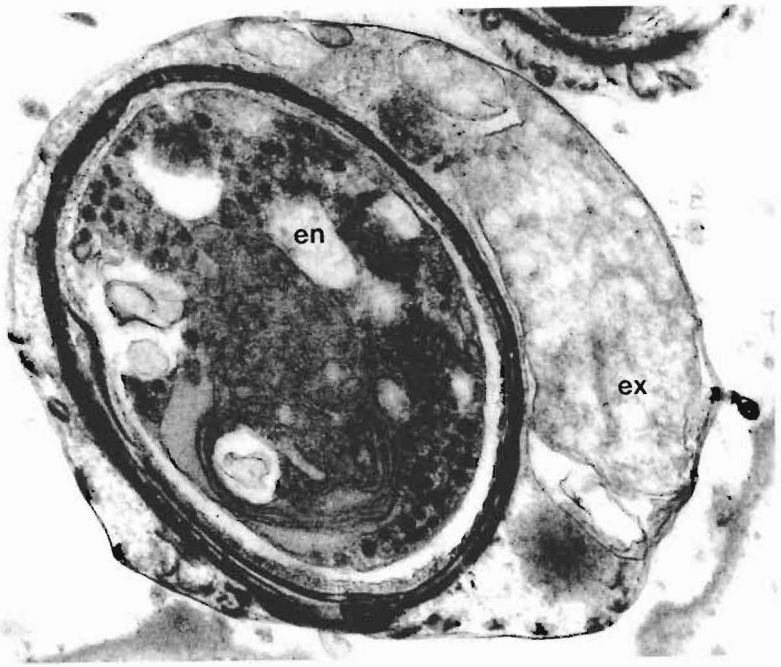


Photo 3.1 - Mature spores (*s*) of *Urosporidium* sp., showing tails, seen under high power on the light microscope.

Measurements show the main body diameter to be approximately 5μm with tail length ranging between 6.5 and 7.5μm. Young spores were pink-stained in these sections, and easily distinguishable from mature spores in that the tail (exospore) was not fully differentiated from the main spore body (endospore) which it still enveloped. Such spores ranged in diameter from 7 to 8μm. A number of plasmodia in the latter stages of sporogony were also apparent and varied both in size, and number of developing spores present. Numbers of spores observed within such plasmodia ranged from 19 to 56. Mature spores congregated towards the outer (host) sporocyst margins whilst developing plasmodia were usually more central.

Photo 3.2 is a transmission electron micrograph of a young spore showing the endospore within the spore wall, surrounded externally by exospore material which would have formed the tail. In more mature spores (Photo 3.3) the site of exospore convergence around the endospore defines the spore's anterior end, where the overlapping lips of exospore cytoplasm also form a circular ridge thereby delimiting the pore region. Some spores were as small as 4μm in diameter but generally conformed with measurements taken on the light microscope. Within the endospore, the golgi apparatus appeared as a "spherule" structure (Ormieres et al. 1973) lying just posterior to the pore region. A single large nucleus was barely apparent towards the centre of the spore, and scattered throughout the cytoplasm were "haplosporosomes" (Perkins 1971) the functional significance of which are unknown. Three layers were observed within the spore wall - a thin inner layer, a clear middle layer about the same thickness, and a dense outer layer (approximately twice as thick as the other two) the components of which were more heavily stained at its base giving the



1µm

Photo 3.2 - Transmission electron micrograph of a young spore showing the endospore (*en*) surrounded by exospore material (*ex*) which have elongated to form the tail.

appearance of two separate layers.

Due to thickness of the spore wall, fully mature spores did not fix or embed successfully for the T.E.M, typically resulting in destruction of endospore material during sectioning. The tail, however, generally remained intact with length measurements ranging from 6 to 7.2µm. Evident in mature tails were mitochondria, large electron dense bodies (usually two), and band-like fibres also

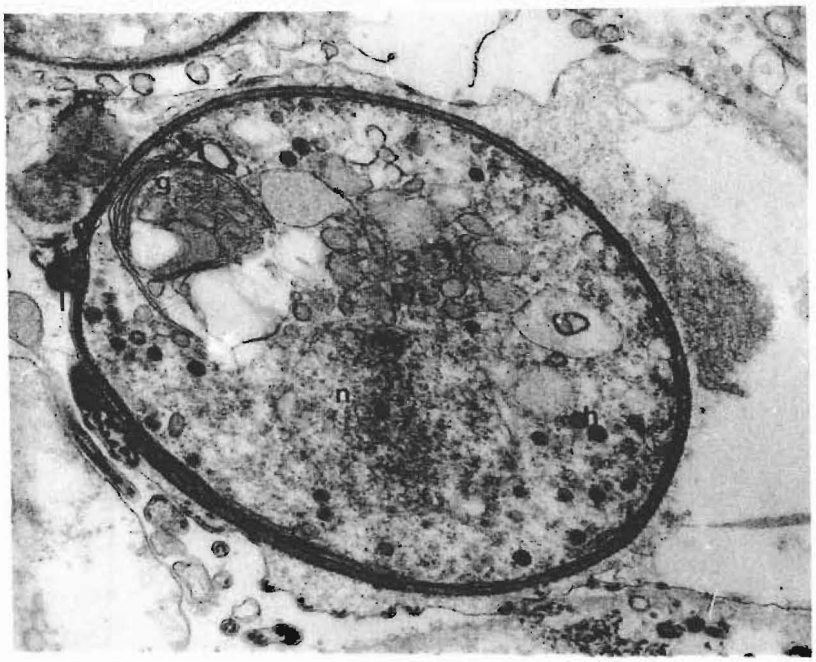


Photo 3.3 - Transmission electron micrograph of a young spore in which can be seen thickened lips (*l*) defining the spore's anterior end, the golgi apparatus (*g*), the large diffuse nucleus (*n*), and numerous "haplosporosomes" (*h*).

described from other species of *Urosporidium* (Perkins 1971; Ormieres et al. 1973). These are all clearly seen in transverse section (Photo 3.4), the bands of fibre appearing as dark semi-circles just under the exospore plasmalemma.

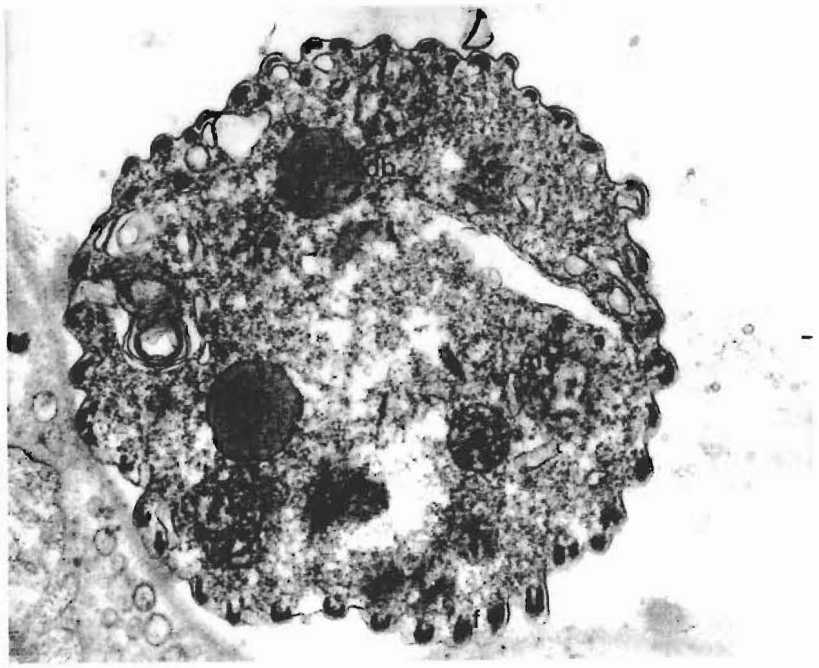


Photo 3.4 - Transmission electron micrograph of a transverse section through the tail of a mature spore. Two electron-dense bodies (*d.b.*) are evident, as are the band-like fibres (*f*) under the plasmalemma, and mitochondria (*m*).

Discussion.

This species of *Urosporidium* generally conforms to the descriptions given for the six other members of the genus. Ormieres et al. (1973) described *U. jiroveci* spores as being between 5.5 to

7.5µm with only one other species - *U. tauricum* - being of equivalent size. Tails of both were also considerably long (*U. jiroveci* 12 to 14µm; *U. tauricum* 15 to 43µm). The remaining four species - *U. fuligonosum U. pelseneeri*, *U. crescens*, and *U. constantae* - are smaller with spores 4 to 5µm in diameter. My species of *Urosporidium* appears to fall within the general size range of the latter described species but differs from both *U. constantae*, and *U. fuligonosum* in that their tails - 10 to 12 µm, and 13 to 15µm respectively (Howell 1967) - are longer, and *U. constantae* is devoid of a ridge around the anterior pore region.

Electron microscopy has been undertaken for only two of the species described - *U. constantae* (Perkins 1971), and *U. jiroveci* (Ormieres et al. 1973). Sections of *Urosporidium* sp. from *C. glandiformis* revealed structures consistent with these studies, however a difference was noted in the Golgi apparatus. For the other two species this consisted of a spherule composed of "anastomosing and convoluted tubular cisternae of variable diameter located at the anterior end of the spore" (Perkins 1971) which was generally considered a "peculiar type of Golgi apparatus" (Ormieres et al. 1973). In my species the Golgi "spherule" resembled more the classic text-book form (e.g. Bloom and Fawcett 1975, pp 53-55) of such structures leaving no doubt as to its true nature. Suggestion has been made (Ormieres et al. 1973) that the Golgi apparatus of Haplosporidia plays an essential role in the later stages of spore morphogenesis.

Although *Urosporidium* sp. from *Cominella glandiformis* is similar in size to both *U. crescens* and *U. pelseneeri*, only the latter is known from sporocysts (parasitising the clam *Abra ovata*, in France).

U. crescens instead parasitises metacercariae found in the blue crab, *Callinectes sapidus*. Due, however, to geographical isolation, and the fact that *Urosporidium* is known to exhibit some degree of host specificity (Bartoli 1974, cited Lauckner 1980), my *Urosporidium* sp. probably constitutes a new species, but this needs to be established from further study both of this and other members of the genus.

Urosporidium sp. is important as a hyperparasite of Cercaria 3 sporocysts in that the host sporocysts are ultimately killed leaving them open to immune responses from *C. glandiformis*. Such immune activation by *C. glandiformis* probably requires energy resources that would otherwise have been available elsewhere. As some authors feel that gigantism is the result, at least in part, of energy surpluses arising from parasitic castration (Sousa 1983) then any changes in energy availability could affect the occurrence of such giant growth in the host snail.

CHAPTER 4.

A COMPARISON OF LABORATORY GROWTH RATES FOR INFECTED AND NON-INFECTED *COMINELLA GLANDIFORMIS*.

Introduction.

The first chapter of this thesis described the way in which fluke infection was positively correlated with snail size in the field, and showed which variables could be used to characterise this relationship. Difficulties arise, however, when interpreting and assigning cause to such correlations. Gigantism (whereby infected snails grow larger and at a faster rate then normal) is one explanation for these correlations, but there are alternatives (e.g. Rothschild 1941) as outlined in the general introductory chapter (pp 7-8). In order to establish if gigantism occurs in *Cominella glandiformis* a study comparing rates of growth between infected and uninfected snails was required. For a direct comparison of such growth rates the procedure should ideally follow the regime first suggested by Lysaght (1941), using only individuals of the same initial size before infection (i.e. of the same age), This also requires, however, that experimental animals are infected in the laboratory.

Where the life cycle of a parasite is known, and larval stages isolated, miracidial infection of naive snail individuals of the same size can be achieved. The only life-cycle known of the three study

parasite species is for Cercaria 1 (*Curtuteria australis*, described by Allison 1979), the miracidial stage of which, however, remains unknown. An alternative to miracidial infection are the sporocyst and redial transplant techniques described by Chernin (1960, 1966), Zischke (1967, 1968), and Jourdane and Theron (1980).

A pilot study employing these techniques on *C. glandiformis* was largely unsuccessful. Snails were anaesthetised with 0.1mg sodium pentobarbitone/ml of surrounding seawater, their shells swabbed with alcohol, and holes drilled through to the underlying gonad/hepatopancreas region. Dissected tissue from either Cercaria 1 (redia) or Cercaria 2 (sporocyst) infections was injected through each hole into recipient tissue, and the hole sealed with sterile plasticine. The majority of the 24 snails which received transplanted tissue from either infection died from operative wounds within one week. Only one recipient (of sporocyst tissue) showed any signs of establishing infection.

The alternative to infecting similar-sized individuals for this study was to follow the growth of field-collected snails under laboratory conditions. Because some snails are infected naturally in the field, before the start of any such study, effects due to parasitism may already be manifest. Similar-sized snails may no longer necessarily be the same age. For this reason a range of snail sizes should be used.

Methods.

Snails were hand collected (as for the field study) from an area of the Avon-Heathcote estuary running parallel to Rockinghorse Road on the South New Brighton Spit. 107 of these, representative of the size range present, comprised the laboratory population. Due to an earlier aquarium failure the study did not begin until mid-summer (January 1988). Each snail was marked with spirit based white-out ("Twink" brand) on which individual numbers were written with graphite pencil, then sealed with water-proof glue. Length (top of spire to tip of siphonal canal) was recorded for each snail (to the nearest 0.02mm) using vernier calipers. Excess water held behind the operculum was expelled by gentle probing, and the animals were blotted dry. Total weights were then obtained on a Mettler H32 balance (accurate to ±0.0003 grams). All snails were housed in perforated 2-litre plastic containers (thirty snails each) under identical conditions in laboratory through-flow seawater aquaria for eighteen weeks. A feeding regime of 2 to 4 (depending on size) cockles (*Austrovenus stutchburyi*) each week was adopted. Greater quantities of food, or more frequent feeding, were avoided as these typically resulted in wastage which quickly fouled the aquaria. Any food remaining uneaten was removed after 24 hours. Individual weights and shell lengths were recorded (using the described procedure) on the 15th, 35th, 54th, 83rd, and 122nd days from the start of the study. On the last day each snail was processed as for the field study (see chapter 1, pp 15-16) with tissue wet and dry weights, shell dry weight, sex, maturity, and type of infection (if any) being recorded.

Statistical Analysis -

Data were principally analysed following the procedure suggested by Kaufmann (1981), also used by Sousa (1983). A specific growth rate (G), defined as the rate of growth divided by size, was calculated from both weights and lengths for each snail. Given intial size (S1) and final size (S2) over the time period (t) (= 122 days), $G = (\ln S2 - \ln S1)/t$. Plots of G against $(S1 \times S2)^{\frac{1}{2}}$ (the geometric mean size) result in straight lines with negative slope, and are essentially plots of the differential equation of a Gompertz curve.

An analysis of covariance (computer package BMDP 1V and 2V, version 1987) was used to test for differences in growth rate between infected and non-infected, male and female, and adult and juvenile snails. ANCOVA tests for differences in the dependent variable (growth rate) when differences among groups in the independent variable (geometric mean size) are taken into account. The dependent variable is tested for equality of group means in a design similar to ANOVA, but the means are first adjusted (through linear regression procedures) for differences in the independent variable between each group (Sokal and Rohlf 1981). Analysis of covariance always tests firstly for equality between slopes of the regression lines (calculated for the dependent variable against the independent). When the equality of slopes is non-significant ($p > 0.05$) this indicates that the dependent variable is correlated to the independent in the same way for both groups.

ANCOVA was also used to compare field data (1748 animals, described in chapter 2) with laboratory data to see if laboratory conditions had any effect on length/weight relationships of the snails. The dependent variables for this analysis were tissue and

shell weights, with length the independent variable for each.

Results.

Eighty-nine of the original 107 snails survived the study period; a breakdown of the group structure is listed in Table 4.1.

MATURITY	SEX	INFECTION	COUNT
Adult	Female	Non-infected	30
		Infected	15
	Male	Non-infected	3
		Infected	12
Juvenile	Female	Non-infected	10
		Infected	1
	Male	Non-infected	15
		Infected	3

Table 4.1 - Group structure breakdown of surviving animals used in the laboratory growth experiment. N = 89.

No increase was recorded in length, and very few snails increased in total weight during the 18 week study period. The apparent lack of

increase in body length could be attributed to caliper erosion of the spire tip through regular measuring. A flat inward thickening of the whorl lip (up to 2mm) across the opercular aperture was, however, noted for most snails suggesting calcium deposition. As thickening in this manner was not observed in field collected animals it can be regarded as abnormal growth, perhaps due to laboratory conditions.

Although G was minimal for the majority of snails with respect to either length or weight, positive growth rates (by weight) were recorded for some juvenile snails. However, regression plots of the specific growth rate (G) against mean geometric size were not significant ($p > 0.05$) for infection (Figure 4.1), sex, or maturity. Because ANCOVA is fundamentally a comparison of regression slopes between groups, little would be achieved by such analysis of this data.

Simple descriptive statistics for infected and non-infected groups showed that each had mean growth rates of zero ± 0.0001 (standard error). Therefore no overall change was apparent in total weight or length during the the eighteen week laboratory period, and no differences were evident between infected and non-infected groups.

ANCOVA for differences in log-transformed tissue weights from animals in the laboratory and those collected from the field showed, however, that for any given length, tissue weight was higher in the laboratory (at the conclusion of the study period). Wet tissue weight was used for the analysis as this gave a better regression against length for field data than did dry tissue weight (appendix pp90-91). Equality of slopes was non-significant only when the data were log-transformed. Regression coefficients for the log-transformed data

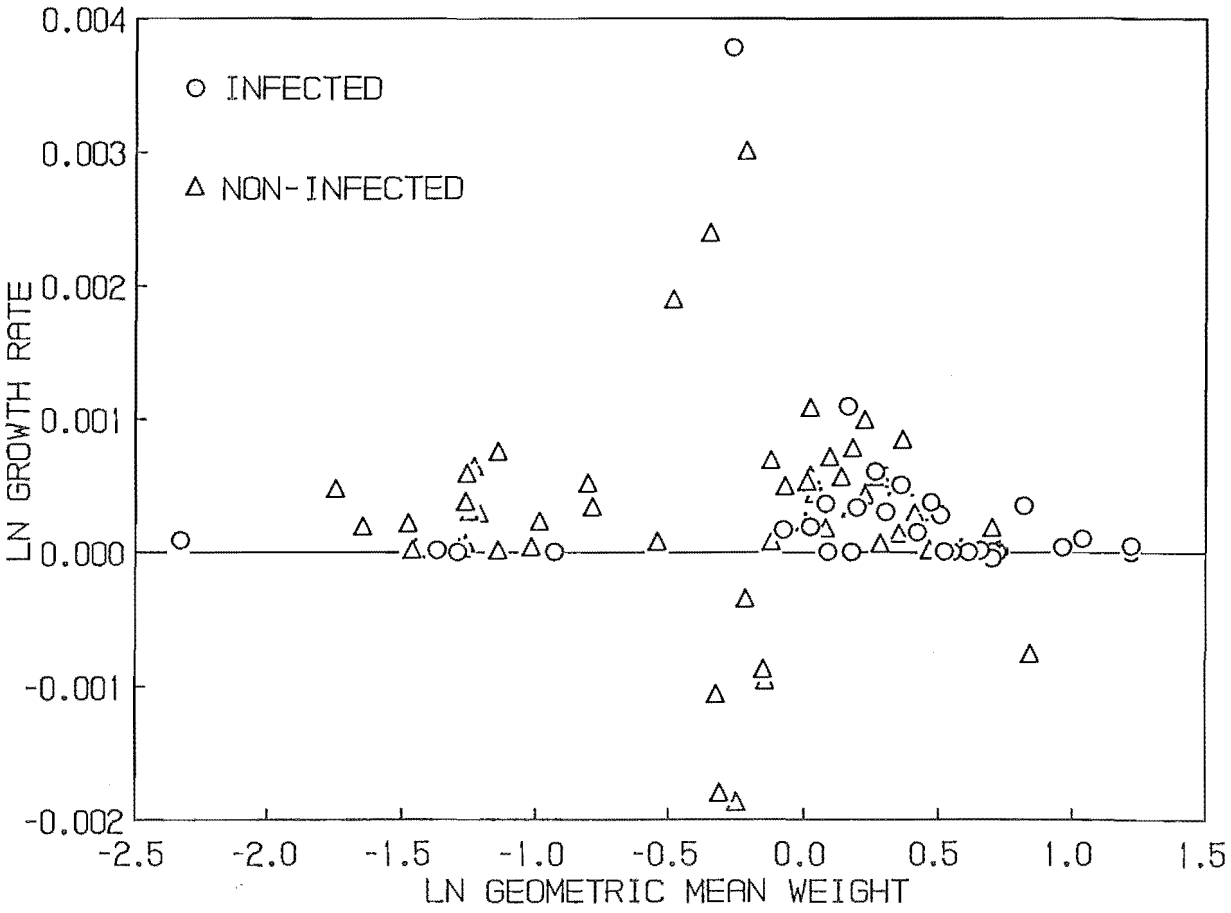


Figure 4.1 - Scatterplot of ln (growth rate) against ln (length) for infected and non-infected individuals kept in the laboratory for eighteen weeks.

Regression coefficients were non-significant for both infected ($r = -0.103$, $p > 0.05$) and non-infected ($r = -0.003$, $p > 0.05$) data. N(infected) = 31, N(non-infected) = 58.

were 2.733 for field data, and 2.910 for laboratory data. Where such coefficients range between 2.5 and 3.5 this usually indicates a relationship whereby weight (w) is equal to a constant (k) multiplied by length (l) to the power of 3 (Calow 1975). That is,

$$w = kl^3$$

Length^3 is taken to represent volume, so a direct (curvilinear) relationship exists between this and weight (Calow 1975).

A significant difference in tissue weights ($p < 0.0001$) was found between the two groups (lab. and field) with respect to length ($F = 99.78$ with 1 df,) but no significant differences were found between infected and non-infected snails. The adjusted means for tissue weight were higher for laboratory animals (Table 4.2). If

	NON-INFECTED		INFECTED	
	Field	Laboratory	Field	Laboratory
TISSUE WET WEIGHT	0.334	0.483	0.346	0.431

Table 4.2 - Adjusted tissue weight means for non-infected and infected *Cominella glandiformis* from the field and the laboratory.

weight-length relationships were simply linear this would mean that tissue weight at any particular snail length was greater in the laboratory than in the field. However the relationship was curvilinear suggesting that such weight differences for laboratory animals became apparent only as length increased.

Analysis of covariance of shell weights between field and laboratory showed a similar, though less significant trend ($p < 0.05$, $F = 3.93$ with 1 df). Adjusted means for shell weight were 0.572 for field data and 0.606 for laboratory data indicating once more that shell weight was greater at each length following maintenance in the laboratory. Regression coefficients for shell weight against length for log transformed data were 2.298 for the field, and 2.488 for laboratory data. Where shell weight is of uniform thickness then a relationship is usually seen whereby $w = kl^2$ (i.e. shell weight is proportional to surface area of the shell) (Calow 1975). Both of the regression coefficients for shell weight fell within the range (1.5 to 2.5) for this relationship, however that for laboratory snails was almost high enough to suggest an association instead with volume. This could indicate that shells of laboratory animals were not of uniform thickness, and shell material may have been deposited abnormally. An unusual thickening of the shell lip, mentioned earlier, also supported this.

Discussion.

Because so little growth was observed in the laboratory, the results obtained from this study are of little use in establishing whether gigantism is responsible for the trend (apparent in the field) of increasing infection prevalence with increasing snail size. Growth was not apparent from either lengths or total weights measured during the eighteen weeks. When separate tissue and shell weights (obtained at the end of the experimental period) for laboratory animals were compared with individuals from the field, weight differences were evident. Laboratory animals were heavier, both in body tissue and shell, than field animals of the same size (length) regardless of infection. This suggests that some growth had occurred during the study. Constant laboratory conditions, and regular food supply, could account for better overall "condition" (e.g. greater fat storage etc.) of snails at all lengths. A more likley alternative is that caliper erosion of the spire tip, and abnormal shell deposition across the opercular aperture (instead of normal extension of the body whorl), precluded any normal length increments from occuring concordantly with growth. The "true" length of each laboratory snail should actually therefore be greater so the observed tissue and shell weights would actually fall within the normal range for snails of such length from the field. Why the growth suggested from this analysis was not apparent from total weights (tissue + shell) of the live animals is not clear. If the blotting of excess water from behind each snail's operculum was not uniform for each of the six measurements over the study period this could, perhaps, have led to a masking effect of the true weights.

Eighteen weeks does not appear to have been a long enough period for study of growth rates of *Cominella glandiformis* under these laboratory conditions. As work did not begin until mid-summer it is possible that a large proportion of growth for the year had already occurred, however the snails were remarkably active when collected and remained so throughout the experimental period. Sousa (1983), in an equivalent field study, reported that another prosobranch, *Cerithidea californica*, commenced growth in late spring, continuing throughout summer, but was dormant by winter when it burrowed into the substratum. He obtained good measures of instantaneous growth rates for this snail using mark-recapture methods for ten weeks also starting mid-summer (last week of June, northern hemisphere). From my field observations, *C. glandiformis* also remains active until at least the onset of winter. The fact that only minimal growth was observed for *C. glandiformis* in the laboratory, over a longer period than Sousa's study, could mean that *Cerithidea californica* is, by comparison, a faster growing snail. Because analysis of tissue and shell weight data suggested that some growth, albeit "hidden", had occurred there is no reason to suspect that *C. glandiformis* should not grow well under laboratory conditions. A repeat of this study starting in spring, with a longer growth period, might yield better results for calculation of growth rates.

Typically, in many gastropod species, differences are found in growth rate both between the sexes, and between immature and mature individuals (Sousa 1983). With respect to sex, faster growth in females results in larger animals with greater fecundity (a function

of size - Hughes and Answer 1982). Greater amounts of growth, as with any animal, would be expected by juveniles. When snails are infected, however, these relationships might not necessarily hold true.

Growth inhibition due to parasitism is commonly recorded, although sex biases also accompany this (Moose 1963; Zischke ad Zischke 1965; James 1979). Sousa (1983) found that some parasitised snails (depending on species of infection) grew at the same rate as uninfected males but considerably slower than uninfected females. Very few cases of actual gigantism have been documented in the laboratory other than Rothschild (1941) and Cheng et al. (1983) - both on marine snails - although temporary gigantism was also observed (for the freshwater snail *Australorbis* (= *Biomphalaria*) *glabratus* by Chernin (1960) and Pan (1965).

Knowledge of how sex and maturity affect the growth rate of parasitised *C. glandiformis* could be as important as establishing the existence of gigantism itself. Such information would allow better understanding of how parasitic castration could be related to giant growth. If, for example, differences are found between the sexes this could relate to a difference in the amount of energy expended by each on gametogenesis (Baudoin 1975). Where snails are castrated, such reproductive energy is available for use elsewhere (e.g. somatic tissue growth). Differences between juvenile and adult snails might relate to the lack of gonad in immature snails. No reproductive energy exists, so none is available for diversion into increased rates of growth.

The means and extent of castration by each parasite, and the ability of snails to withstand infection could also be associated with

expression of gigantism, and growth rate overall, of *C. glandiformis*. Information pertaining to this was documented in the histology chapter (chapter 2) and will be discussed in the context of causation of gigantism, along with field and laboratory data, in the general discussion chapter to follow.

GENERAL DISCUSSION AND CONCLUSION.

The aim of this study was to investigate possible relationships between the size of *Cominella glandiformis* and its parasite fauna. Whenever positive correlations have been reported between host snail size and parasite prevalence, the phenomenon of gigantism has also been discussed (e.g. Rothschild 1936; Lysaght 1941; Cheng 1971; Sousa 1983; Minchella 1985).

Several studies have attempted to relate such correlations to gigantism with varied results. In the laboratory, Chernin (1960) and Pan (1965) found that juvenile *Biomphalaria glabrata* parasitised by *Schistosoma mansoni* grew faster (and larger) than normal until maturity from which time both size and growth rates were surpassed by controls (this was a temporary form of gigantism until host maturity). Cheng (1971) and Cheng et al. (1983) found that the greater weights of many trematode-infected snails were due to increased calcium deposition in the host shell - possibly a function of parasitic stress. They also recorded greater host body tissue weights for one trematode species providing further indirect evidence for increased tissue growth. Field studies, mainly using capture-recapture methods, have mostly found that although positive correlations between infection and snail size were apparent, parasitised snails grew slower than their non-infected counterparts (Cannon 1979). Rate of growth also varied according to sex and reproductive maturity of the host individual (Moose 1963; Sousa 1983).

My study showed that positive correlations existed in the field between size of *Cominella glandiformis* and parasite prevalence.

Details of this were given in chapter 1. The existence of gigantism, however, seemed unlikely as infected individuals did not comprise a group convincingly separate from uninfected based on measured size variables. Instead, both appeared to belong to only a single population with approximately normal distribution. Rothschild (1941) listed a number of alternatives to gigantism where similar correlations might also be evident. These were described in the general introduction and included younger (smaller) snails being more attractive to miracidia, infection being lethal to younger snails, and older (larger) snails having greater time and/or opportunity to pick up infection. The last of these is the most likely for *C. glandiformis*. As larger snails of both sex were more commonly found higher on the shore, where infection prevalence was also greatest, this seemed to suggest that individuals probably move higher on the shore as they age, into an area where miracidial exposure might also be greater.

One alternative rarely mentioned in the literature is that infection could increase the life-span of infected snails whilst leaving growth rates unaffected. Positive size-infection correlations would result as such snails would continue to grow, attaining sizes outside the normal range of uninfected snails. Little is known of the mechanisms that influence longevity in molluscs and in many gastropod species a specific age of mortality (i.e. modal age of death in a population) is not apparent (Comfort 1957). In many species growth ceases or is slowed at the onset of reproduction (Comfort 1957); castration could account for failure of such growth checks to occur. Such longevity could not be described as gigantism, as for giant growth to occur the infected snails must grow faster, ultimately

becoming larger, than uninfected during some stage of their development.

To establish the existence of gigantism within a population it is necessary to compare the growth rates of infected and uninfected individuals. According to Lysaght (1941) this should be undertaken using snails of the same initial size so as to obtain a common baseline from which to work. However this is not essential when instantaneous growth rates (Kaufmann 1981) can be calculated for snails of different sizes. These can then be used to directly plot and compare the growth curves of infected and uninfected individuals. Sousa (1983) used this method successfully in the field, and I employed a similar method for use with *Cominella glandiformis* in the laboratory (chapter 4). As the results from my study were inconclusive the question of gigantism in *C. glandiformis* remains for the present unanswered. However, the suitability of *C. glandiformis* as a candidate for giant growth can be examined from consideration of current theory concerning such growth phenomena.

Because each snail/fluke combination is unique, with different effects on the host, there is little basis for comparison from one study to another. Generalisations have been attempted (e.g. Sousa 1983; Minchella 1985) in order to predict the outcome of any given snail-parasite interaction. These mostly invoked cost-benefit strategy, resulting in three conflicting hypotheses which each make a number of assumptions and predictions. These hypotheses will be discussed in turn but should each be considered against a background of alternative life-cycle strategies utilised by short-lived (typically freshwater) and long-lived (typically marine) snails. Short-lived snails usually only reproduce in one season, expending

little energy on repair mechanisms but considerable amounts on reproduction (gamete production) (Minchella 1985). Long-lived snails spread reproductive effort over a number of years with high levels of repair and maintenance.

The three hypotheses to be considered were first put into perspective by Minchella et al. (1985) who named and categorised each:

1. **The energy allocation hypothesis.** Increased growth is a side effect or non-adaptive consequence of castration, reproductive energy freed being channelled into somatic and/or shell growth.

2. **The prudent-parasite hypothesis.** Abnormal host growth is considered a parasite-induced phenomenon if associated with increased host survivorship providing a more stable resource for the parasite.

3. **The temporal-compensation hypothesis.** Enhanced growth is a host counter-adaptation if it allows increased survivorhip of the host which can outlive the infection and reproduce.

According to the first hypothesis, the energy allocation hypothesis, parasitic castration will release only that energy immediately available for reproduction - much in short-lived snails, less in long-lived, nil in juvenile individuals of either type. Gigantism may occur in adult individuals where castration is complete but parasite nutritional requirements are not particularly heavy. In these cases an energy surplus could occur resulting in increased host somatic growth. Such surpluses seem likely only in adult short-lived snails. Castration of long-lived snails will result in only small quantities of free energy by comparison with short-lived hosts - enough energy may be available to meet parasite needs but if not, and if energy demand exceeds availability, then the parasite might instead drain energy from host somatic growth and repair. This is the

prediction made for all juvenile snails regradless of life-span. A consequent stunting of growth in both juvenile and long-lived adult snails might be expected.

The **prudent-parasite hypothesis** assumes parasite control of host energy resources. Castration releases reproductive energy which the parasite channels initially into non-reproductive processes such as host fat storage and somatic growth resulting in greater host size. Assuming fecundity of the parasite is proportional to host body mass, then host size and parasite fecundity are positively correlated (Baudoin 1975), the parasite deriving direct benefits. As the parasite can always match its own growth to energy availability, surpluses are unlikely to occur.

The **temporal-compensation hypothesis** was developed by Minchella (1985) and assumes the host retains control of its energy resources. According to Minchella, resistance to parasite invasion should always be the first "option" to a snail, but will only evolve in a population if:

(Probability of being infected) x (Costs of being infected) > Cost of host resistance

Where costs outweigh the benefits then low-cost alternatives involving temporal compensation will instead evolve. High costs at the expense of reproduction or other vital functions, low probability of infection, or counteracting forces by two or more parasites will tend to select against the development of any resistance.

For gigantism to occur under the temporal-compensation hypothesis,

i. infection must result in complete inhibition of host reproduction with castration being indirect with little or no irreversible physical damage to gonads, and

ii. the negative efects of parasitism must be short-term, the host having an appreciable chance of outliving infection.

Reproductive energy is released due to host castration, and used for snail somatic growth at the expense of parasite growth, so damage to the host is minimisd. The snail survives infection with gonad function recoverable for delayed reproduction. As only long-lived (usually marine) snails have any appreciable chance of outliving infection, this counter-adaptation would be expected only in such snails. Should reproduction continue, even to a limited degree, then it is more advantageous for an individual to use its resources instead for gamete formation.

If a high probability exists that infection will result in the total reproductive "death" of a snail (i.e. irreversible physical damage to gonads) then an alternative to temporal-compensation - fecundity compensation - may be apparent. Fecundity compensation is usually evident from significant increases in egg-laying before patency of infection (i.e. after miracidial penetration but before cercarial production by the parasite), and theoretically can occur in both long- and short-lived snails. Individuals utilising this strategy are guaranteed some measure of reproduction. Rapid bursts of growth to adulthood by near-mature snails can be associated with fecundity compensation (Thornhill et al. 1986) allowing such snails to also benefit; this was used to explain the temporary gigantism (mentioned earlier) reported for *Schistosoma* infected *Biomphalaria glabrata*.

As *Cominella glandiformis* is a long-lived marine species, the energy surpluses required under the energy allocation hypothesis, for gigantism to occur, are unlikely unless parasite loads are exceptionally light. Histological examination (chapter 2) of parasitised tisue showed that infection in the majority of cases was always quite severe. Less pronounced infection was recorded only for a few snails parasitised by Cercaria 1 but as this parasite species was found to preferentially invade gonad (rather than hepatopancreas), which was always severely damaged (usually detroyed), the effect was such that energy surpluses were still unlikely.

Gigantism could exist in *C. glandiformis* under either of the two remaining hypotheses - the temporal-compensation hypothesis, and the prudent-parasite hypothesis. Adherence to the first assumption required by Minchella (1985) for temporal-compensation (i.e. complete but reversible castration) precludes the use of this strategy by *C. glandiformis* harbouring Cercaria 1 infections. From chapter 2, such infection physically (directly) destroyed gonad leaving regeneration seemingly impossible. Incomplete castration was found in some Cercaria 2 infections but the extent to which actual reproduction was possible is questionable. The temporal-compensation hypothesis is only valid for such infection if the ova (apparently intact) observed concurrent with infection were capable either of full development or of being externally deposited. Although castration by Cercaria 3 infection was both complete and indirect, such infections were hyperparasitised to such an extent that host resources were unlikely to be available for additional somatic tissue growth.

The premise that parasitised snails are capable of limiting the progress of infection merely by making reproductive energy unavailable

for parasite use seems remarkable. The energy available from parasitic castration is itself usually not enough to meet parasite nutritional requirements (Becker 1980, cited Minchella 1985) so additional use is made of host resources (e.g. digestive gland). Although some energy would be lost to the parasite if reproductive effort was instead placed into host tissue growth, I feel that the parasite is as capable of taking nourishment from such tissue as from gonad. Net gain to the host would consequently be minimal. Minchella (1985) cited examples of self-cures, whereby some individual snails outlived infection, but these are rare for most snails (Sousa 1983) and no evidence of such was found for *C. glandiformis* throughout either field or laboratory studies. Surgical transplantation experiments (Chernin 1966, Jourdane and Theron 1980) have shown that given fresh resources a parasite can produce numerous successive generations of daughter redia or sporocysts, and are therefore quite capable of matching reproduction both to the energy resources available and to life-span of the host. The second assumption for temporal-compensation (short-lived infection), therefore, would be rarely met.

There can be little doubt as to the benefits derived by parasites infecting larger hosts. As redial/sporocyst and cercarial production are matched to resource availability (Baudoin 1975), a larger host equates with greater probability that cercariae from any one infection will contact a definitive host. Where gigantism cannot be attributed merely to transient energy surpluses (i.e. the energy allocation hypothesis) there would seem a good case for the prudent-parasite hypothesis. Regardless of "strategy", however, any increase in individual host survivorship will also increase survivorship of the

parasite. The alternative to gigantism, mentioned earlier, whereby infected snails live longer than uninfected is an example of this.

In conclusion, no evidence for gigantism was demonstrated for *C. glandiformis* regardless of infection. Field data indicated that the correlation found whereby infection prevalence increased with host size could probably be accounted for by one of the described alternatives to gigantism. Histological examination of infected tissue, when considered against current theory, discounted the possibility of gigantism in *C. glandiformis* due to host adaptation, or transient nutritional surpluses for at least two of the parasite species (Cercariae 1 and 3). Gigantism might be available as a host adaptation to snails parasitised with Cercaria 2 infections if total reproductive inhibition occurs (i.e. the ova observed must be demonstrably non-viable). Aside from this, if future work indicates that giant growth does occur in *C. glandiformis* then this can most likely be attributed to parasite adaptations.

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APPENDIX.

SCATTERPLOTS AND CORRELATION STATISTICS FOR *COMINELLA GLANDIFORMIS* COLLECTED FROM THE FIELD.

Data used for these plots are those from chapter 2 (field study). The scattergrams and accompanying statistics were generated by the computer package BMDP (version 1987). Numbers in the plots are the frequencies of points plotted at the same positions. For counts greater than 9, A denotes 10, B denotes 11, etc., and an asterisk indicates a frequency of 36 or more (BMDP manual, 1985). Y's on axes indicate points of intersection by line of best fit.

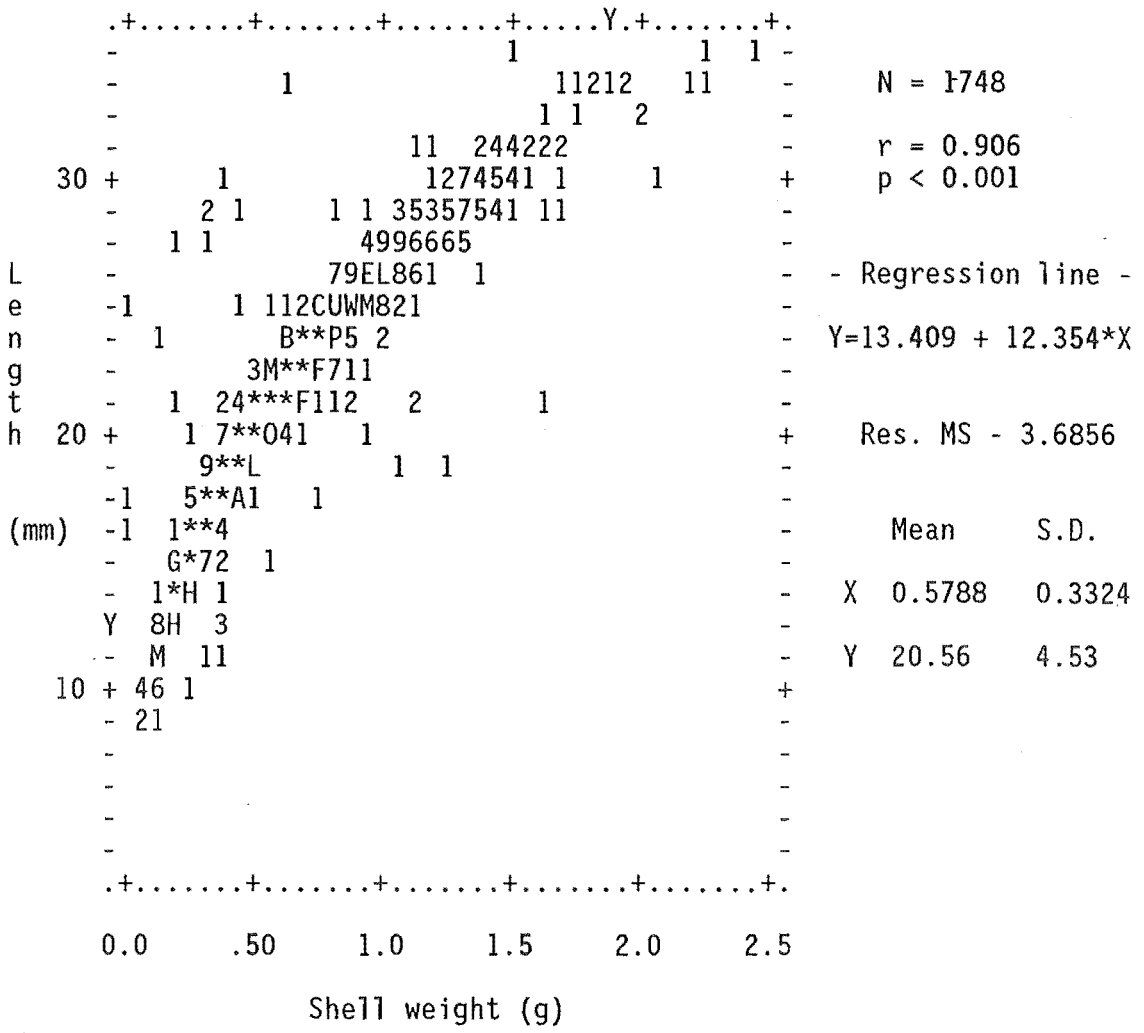

Figure A.1 - Scattergram and statistics of *Cominella glandiformis* from the Avon-Heathcote estuary - LENGTH (mm) vs SHELL WEIGHT (g).

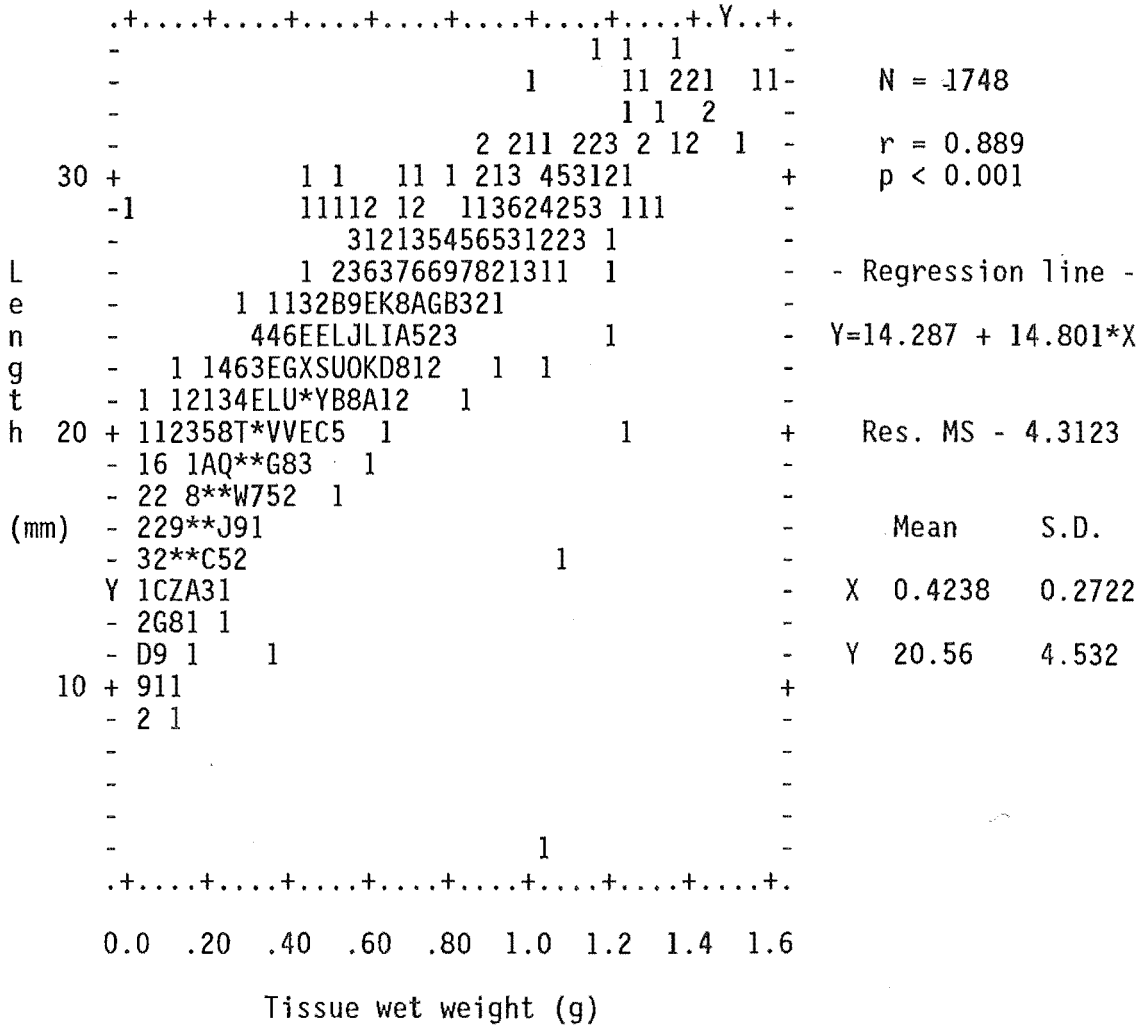

Figure A.2 - Scattergram and statistics of *Cominella glandiformis* from the Avon-Heathcote estuary - LENGTH (mm) vs TISSUE WET WEIGHT (g).

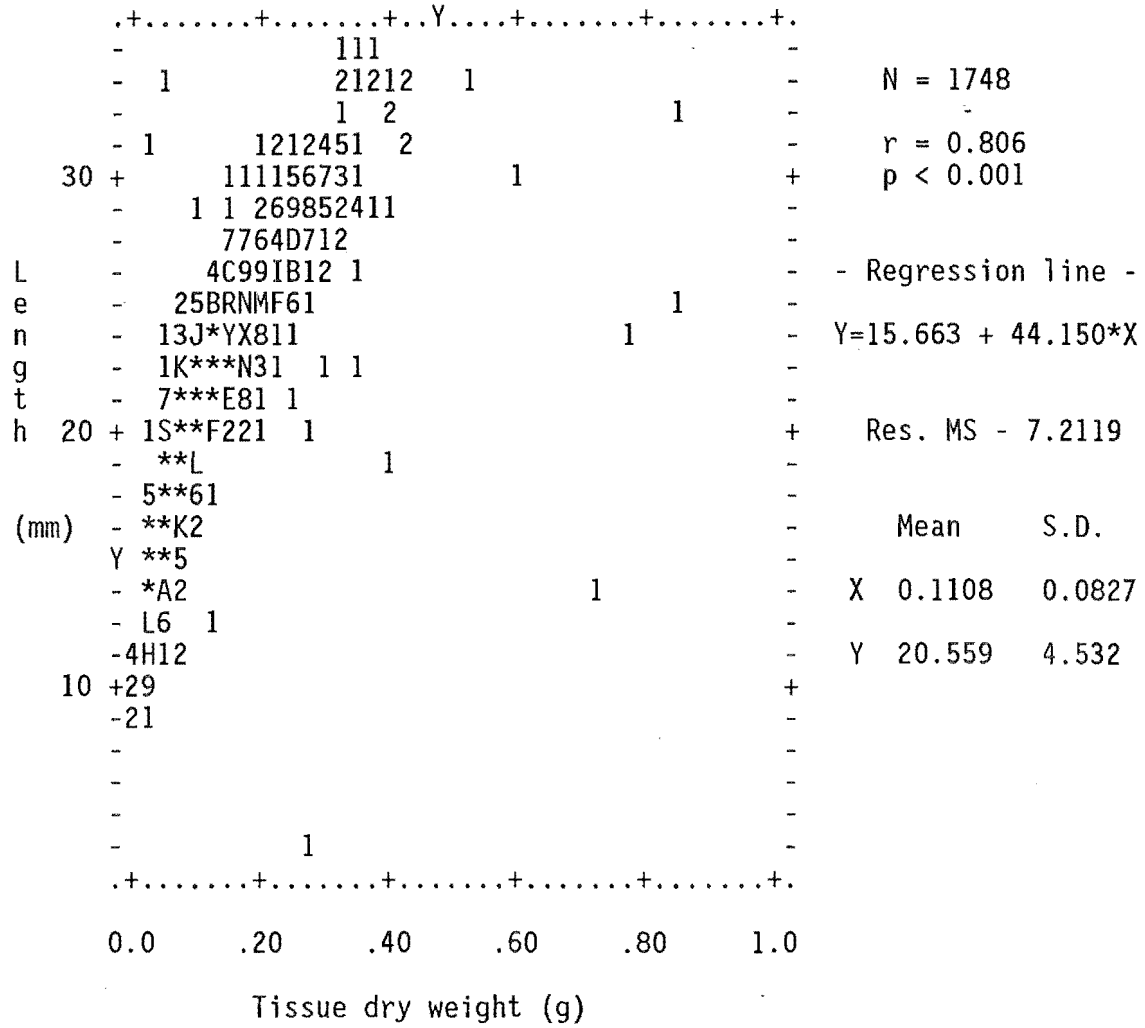

Figure A.3 - Scattergram and statistics of *Cominella glandiformis* from the Avon-Heathcote estuary - LENGTH (mm) vs TISSUE DRY WEIGHT (g).

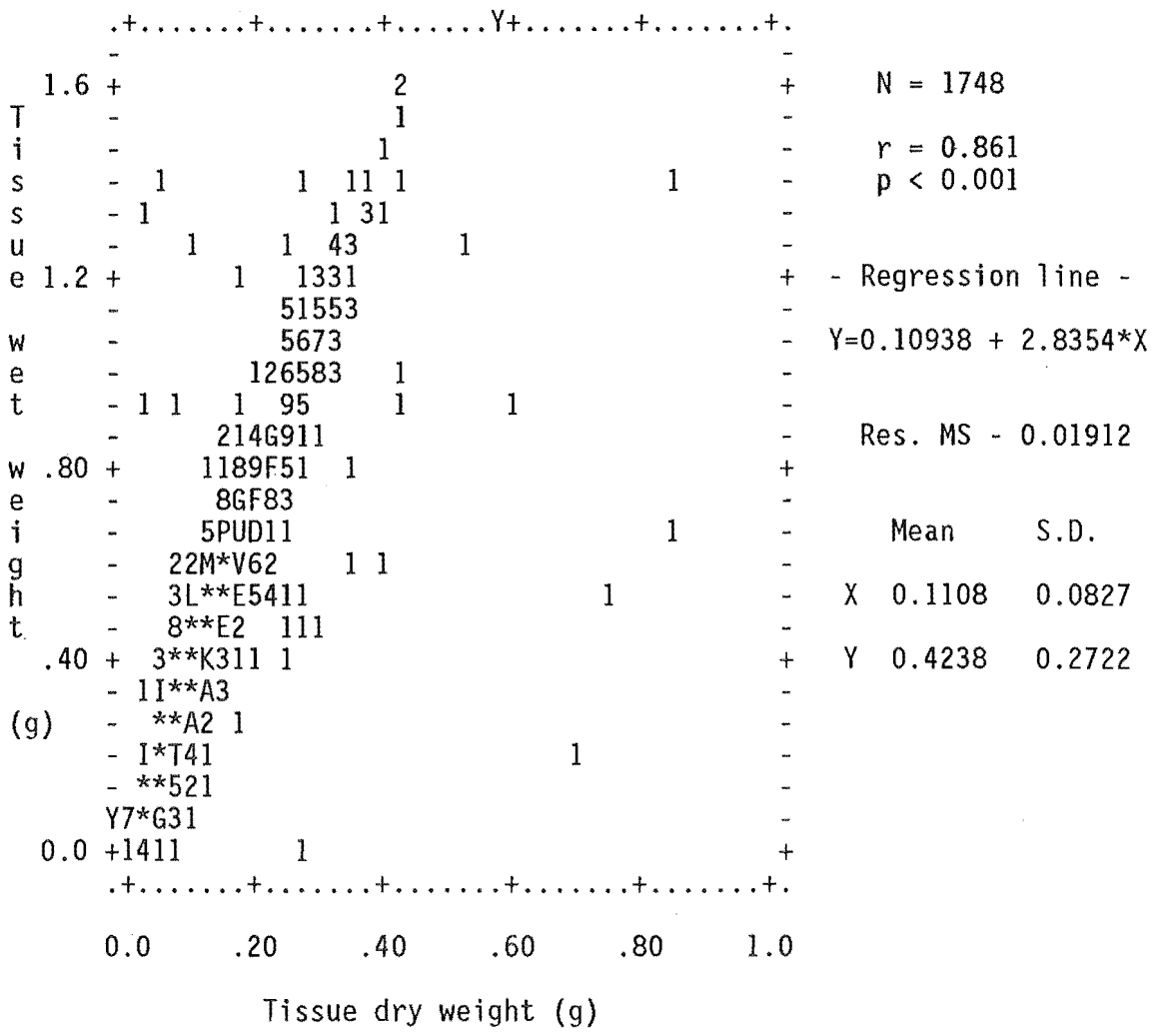

Figure A.4 - Scattergram and statistics of *Cominella glandiformis* from the Avon-Heathcote estuary - TISSUE WET WEIGHT (g) vs TISSUE DRY WEIGHT (g).

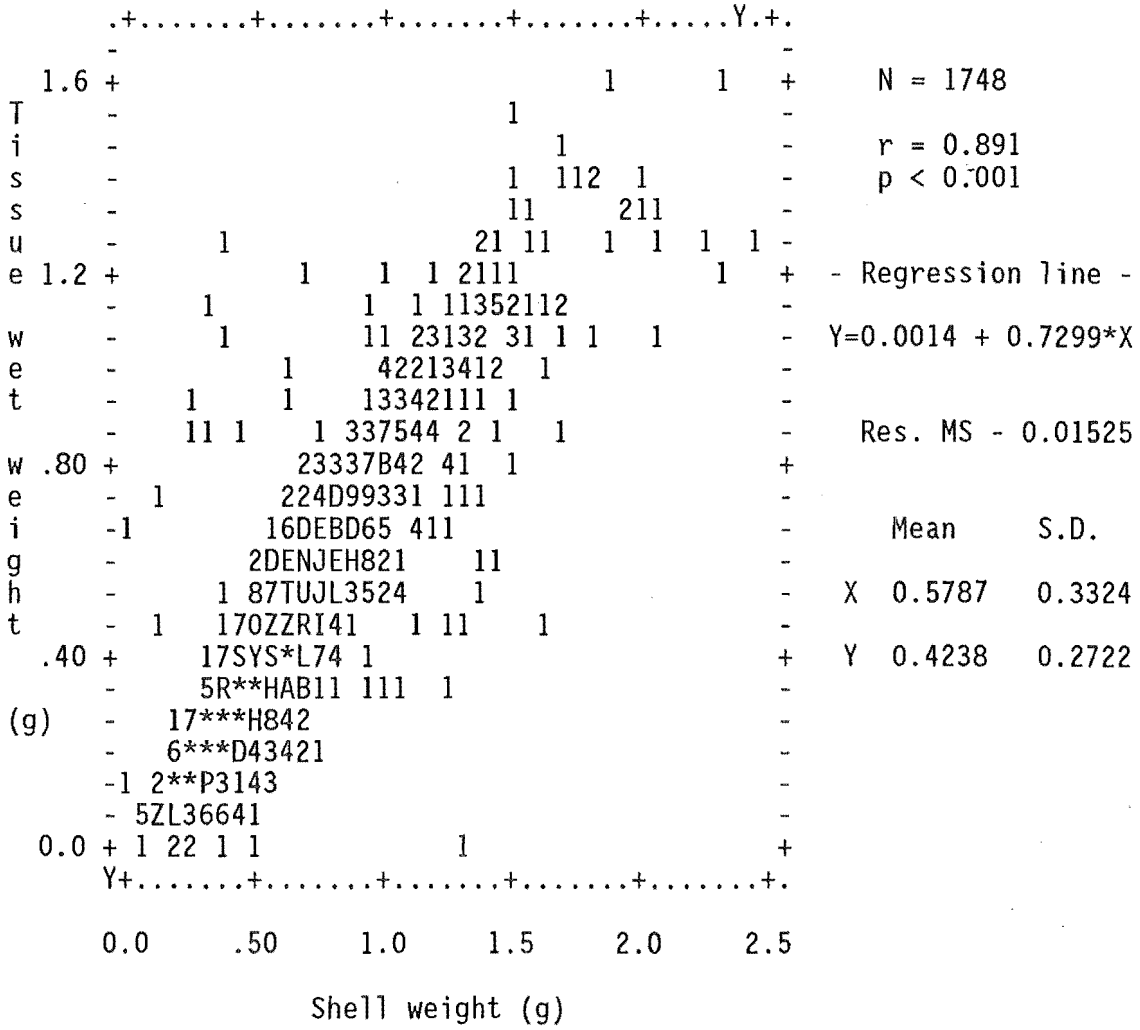

Figure A.5 - Scattergram and statistics of *Cominella glandiformis* from the Avon-Heathcote estuary - TISSUE WET WEIGHT (g) vs SHELL WEIGHT (g).

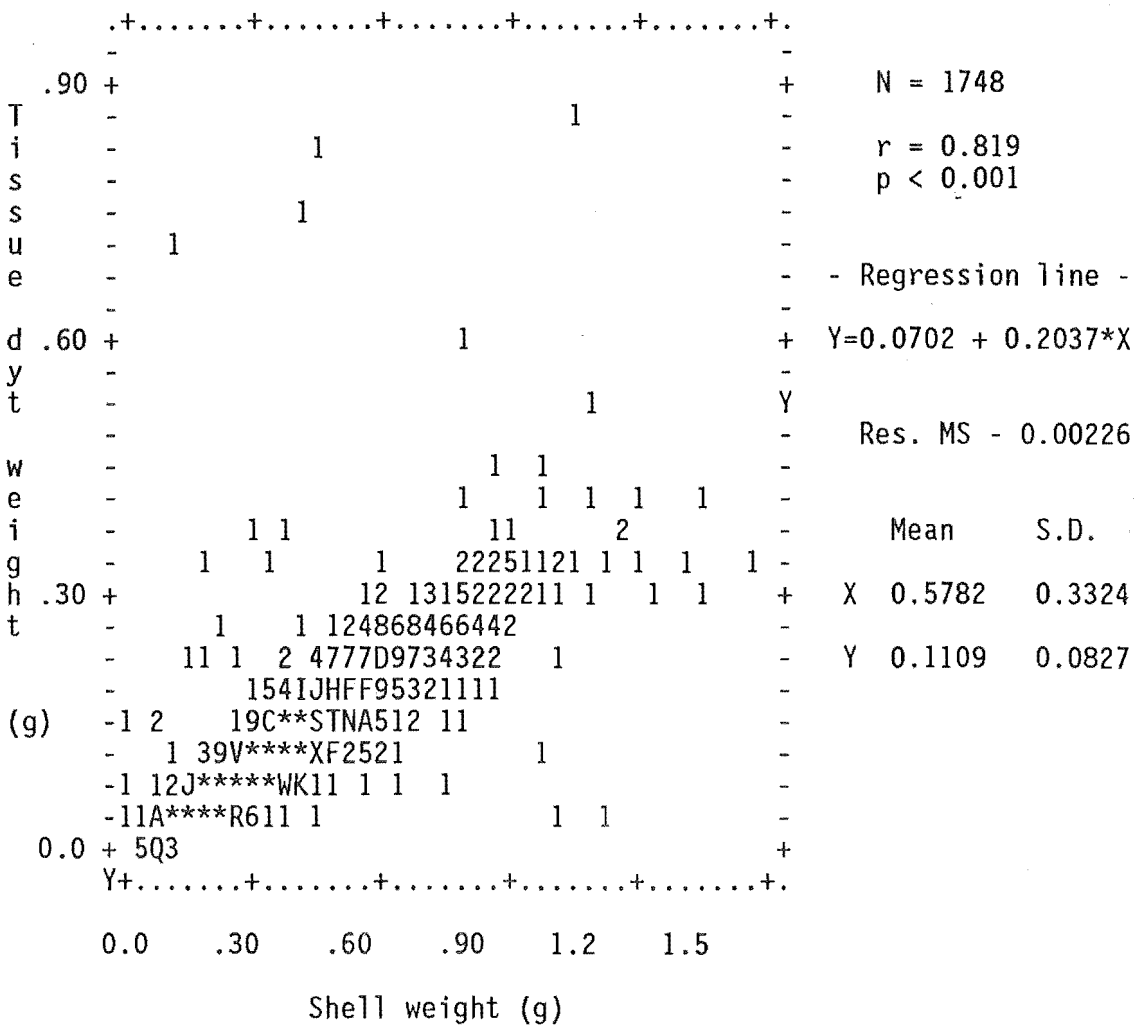

Figure A.6 - Scattergram and statistics of *Cominella glandiformis* from the Avon-Heathcote estuary - TISSUE DRY WEIGHT (g) vs SHELL WEIGHT (g).